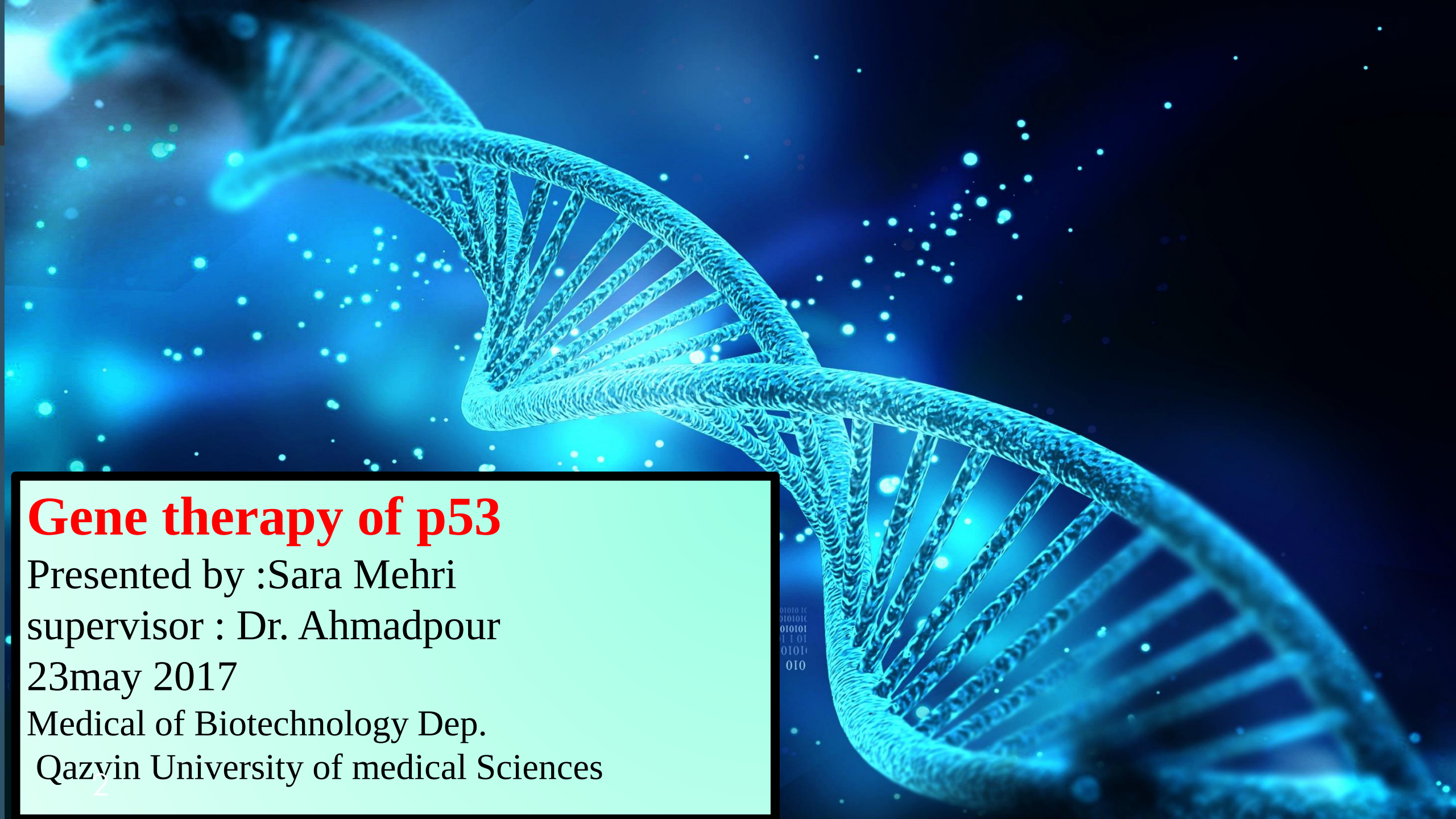
In the name of God



Gene therapy of p53

Presented by :Sara Mehri
supervisor : Dr. Ahmadpour
23may 2017
Medical of Biotechnology Dep.
Qazvin University of medical Sciences

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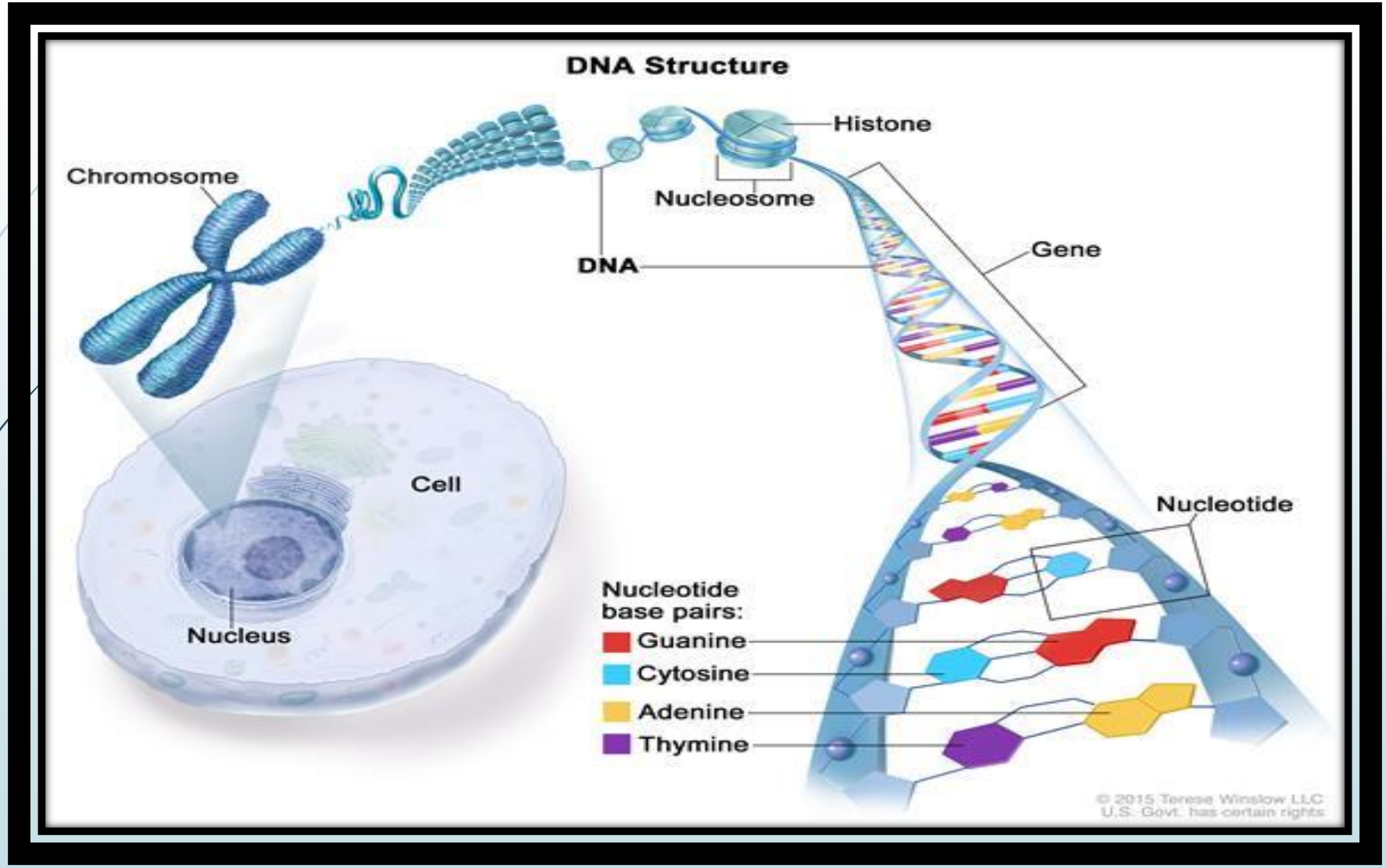
Genes



Captured from : www.cancer.gov

Tumor suppressor p53

- the guardian of the genome
- essential for conserving genomic stability by preventing mutation
- its mutation and inactivation are highly related to all human cancers.(2)

Function of tumor suppressor p53 in cancers

- formation of **p53 tetramers** is needed to act as a transcription factor of several target genes.(3)

- **posttranslational mechanisms** abrogate it’s functions and stability(2)

Function of tumor suppressor p53 in cancers

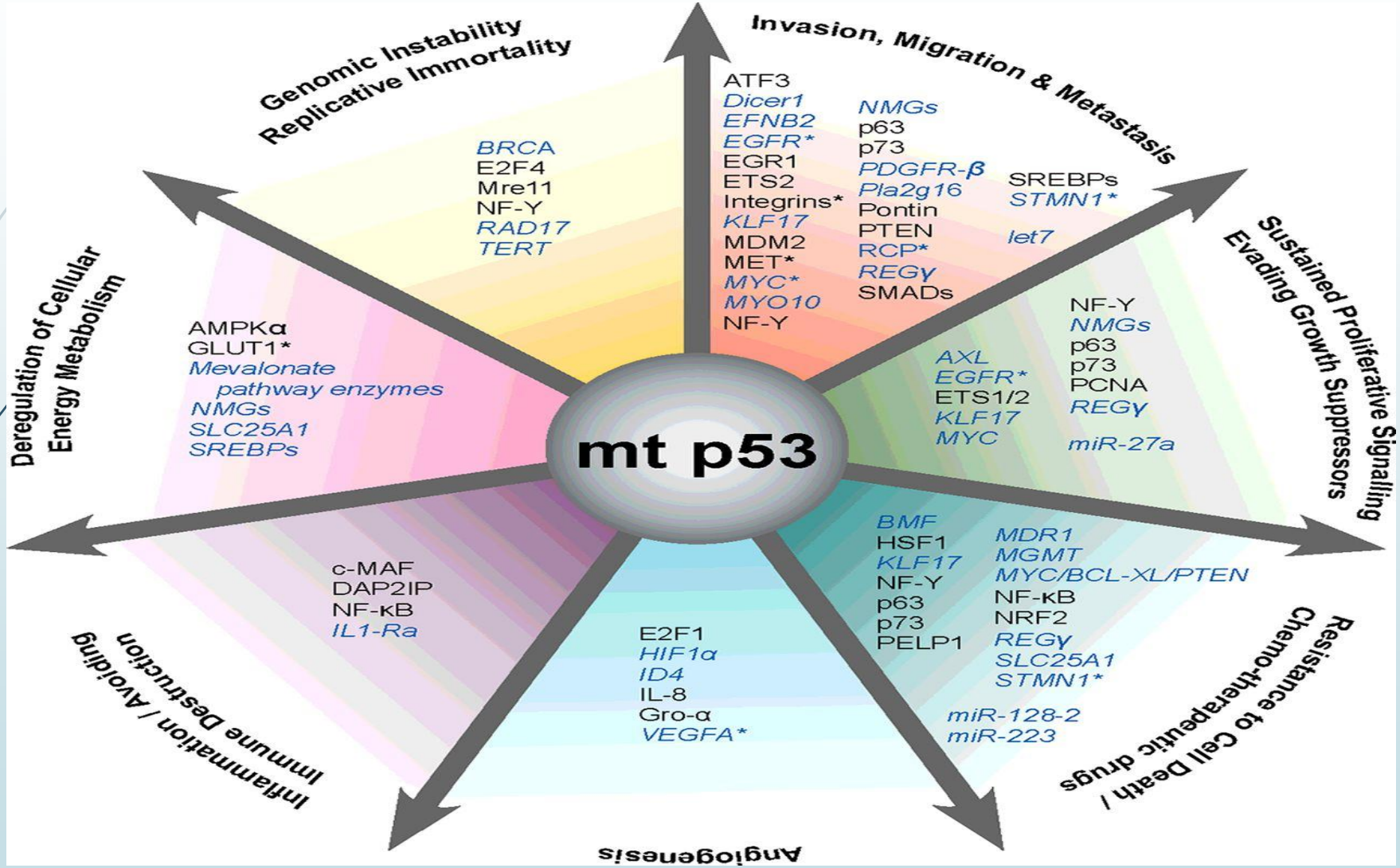
- **missense mutations** of the TP53 gene is the most common mechanism of it's inactivation.

- The majority of these mutations take place in the **DNA binding domain** (DBD).(3)

Function of tumor suppressor p53 in cancers

- the correlation of its inactivity and malignant development is highlighted .(2)
- Cancers often deactivate p53, because:
- Trigger:
  - Cell growth arrest
  - Apoptosis
  - Autophagy
  - Senescence
- Impede:
  - Cell migration
  - Metabolism
  - Angiogenesis

Function of tumor suppressor p53 in cancers



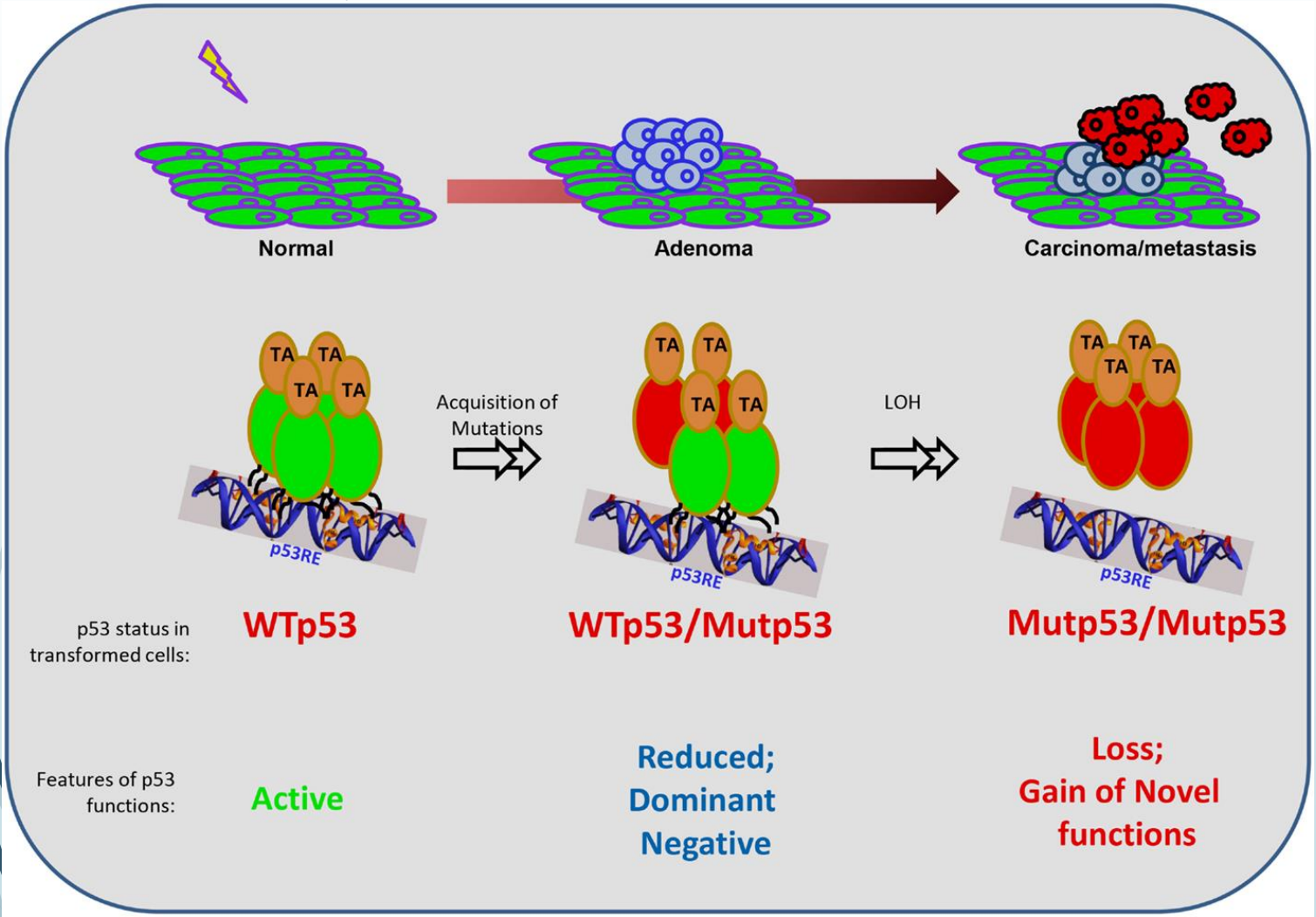
Captured from: http://www.biochemsoctrans.org

Function of tumor suppressor p53 in cancers

- **trans dominant inhibition** by mutant p53 causes impaired function.

- the dominant negative effect of mutant p53 rise to a **critical barrier** to utilizing wt-p53 for cancer gene therapy(3)

Function of tumor suppressor p53 in cancers



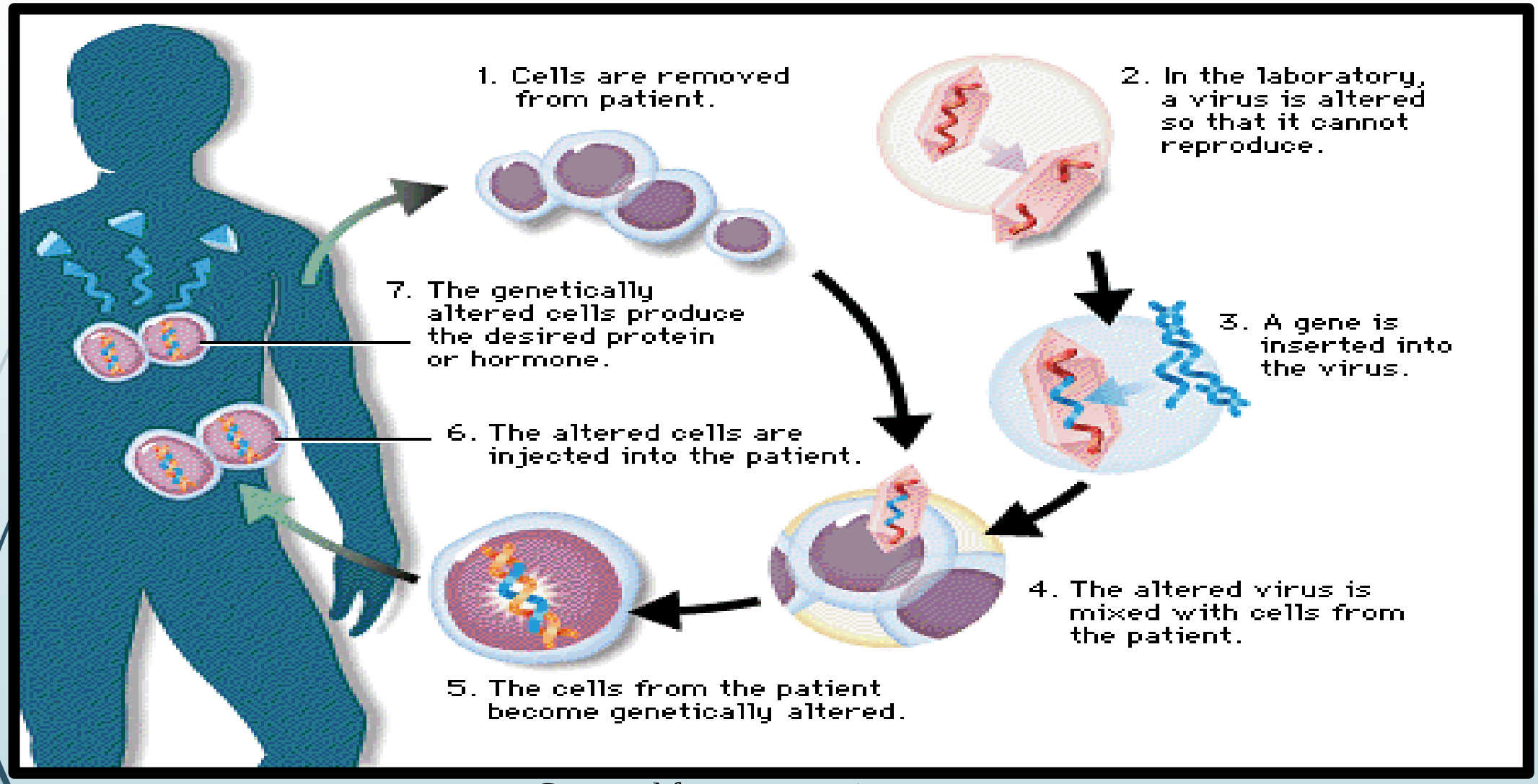
trans dominant inhibition(3)

What is Gene Therapy?

- one of the most rapidly developing fields of molecular medicine.

- It allows simple transfer of genetic methods aimed at correcting pathological processes into clinical practice(4)

What is gene therapy



Captured from: www.pinterest.com

Type of gene therapy

Monogenic gene therapy

- Provides genes to encode for the production of a specific protein

Suicide gene therapy

- Provide ‘suicide’ genes to target cancer cells for destruction

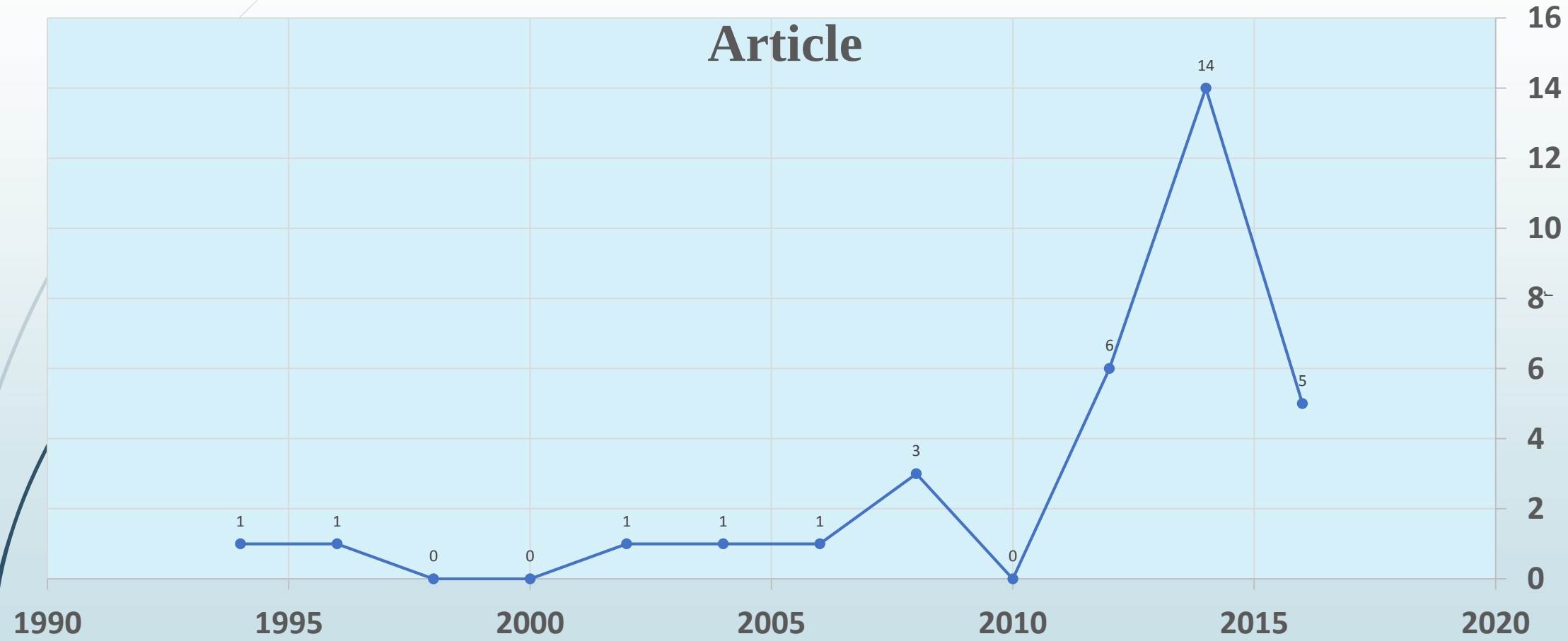
Antisense gene therapy

- Provides a single stranded gene in an ’antisense’ orientation to block the production of harmful proteins.(6)

Gene therapy targets

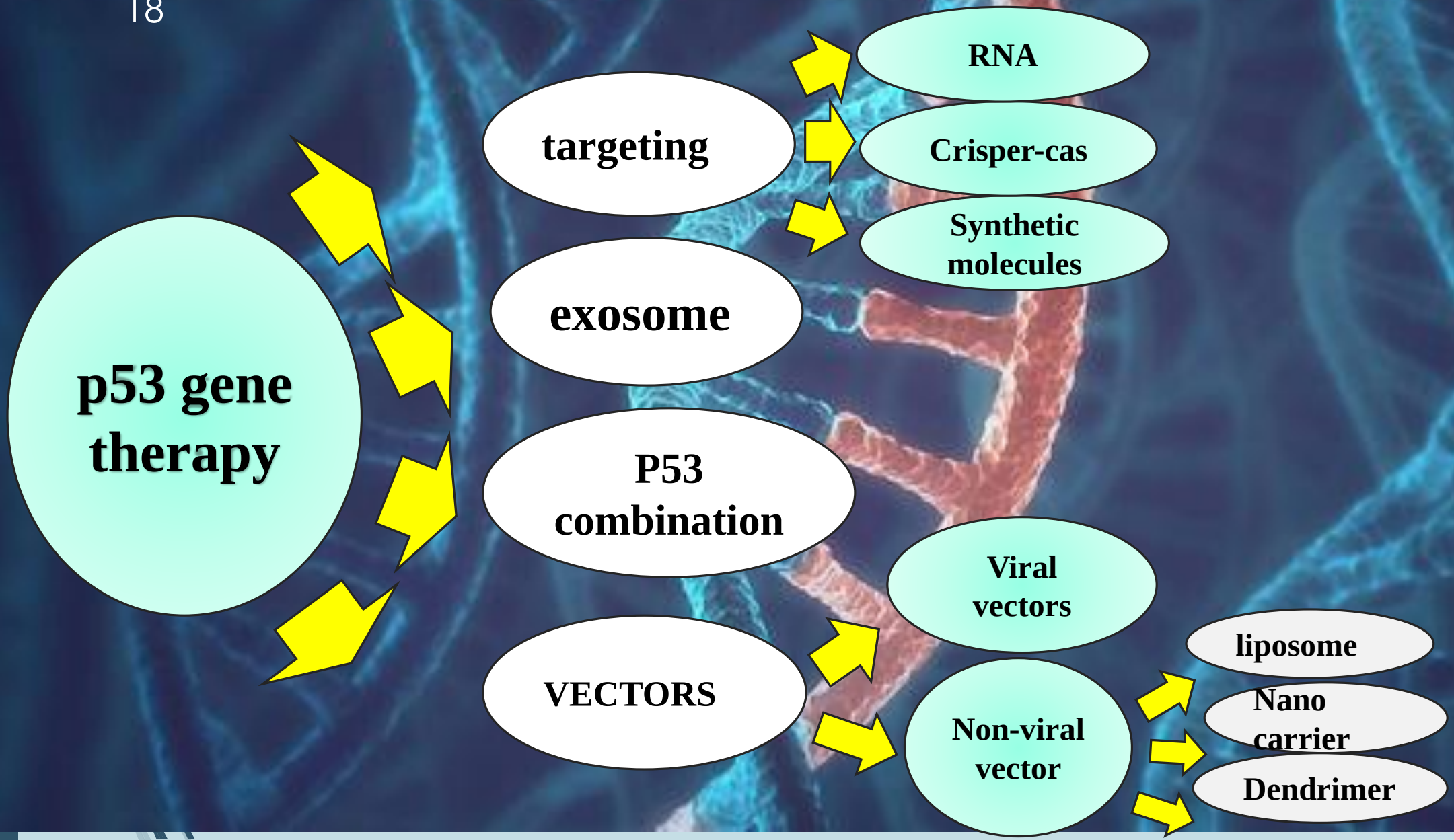
- Germ line gene therapy
- Somatic cell gene therapy(6)

p53 gene therapy /1996 to 2017



Google scholar & www.ncbi.com /
last update:10 April 2017

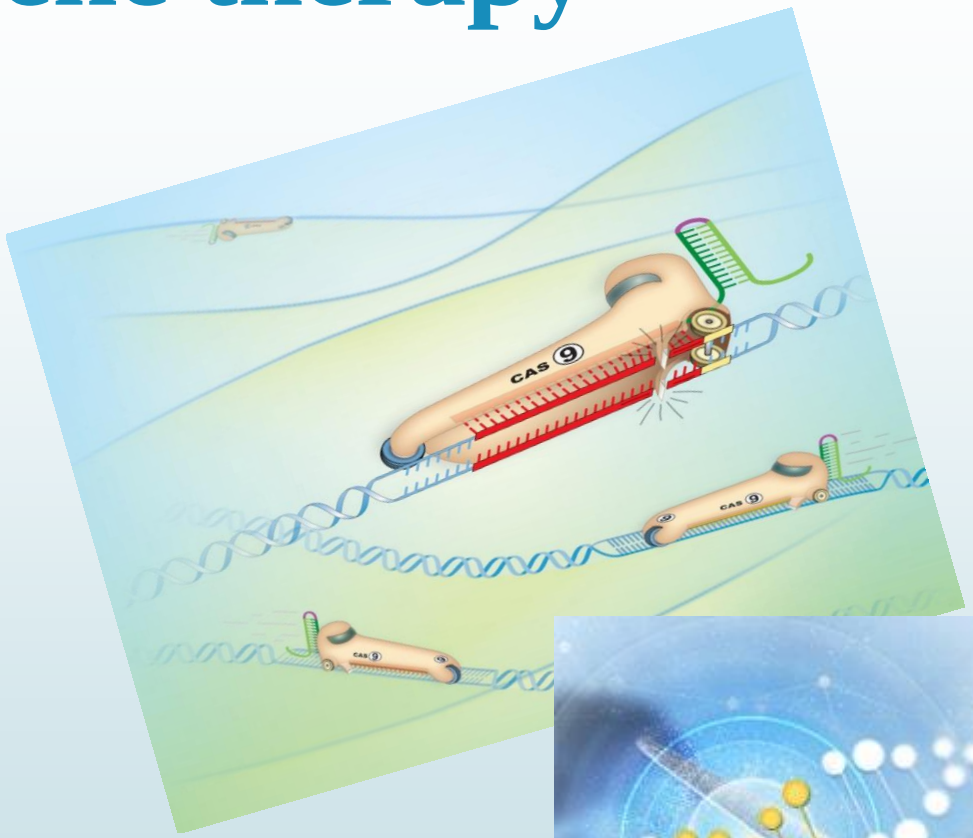
Discussion


p53 gene therapy
targeting
exosome
P53 combination
VECTORS
RNA
Crisper-cas
Synthetic molecules
Viral vectors
Non-viral vector
liposome
Nano carrier
Dendrimer

Types of gene therapy

Targeting

- synthetic molecules
- RNA
- Crispr/ cas



investigations on P53 gene therapy

Type of gene therapy	Type of targeting	year	Related articles
targeting	synthetic molecules	2014	Targeting p53-MDM2-MDMX Loop for Cancer Therapy
		2014	p53 as a target for the treatment of cancer
		2008	Mutant p53 targeting by the low molecular weight compound STIMA-1
		2007	Targeting the p53 Family for Cancer Therapy: ‘Big Brother’ Joins the Fight
		2006	Strategies for therapeutic targeting of the p53 pathway in cancer

Targeting>>MDM2 and MDMX

Published in final edited form as:
Subcell Biochem. 2014 ; 85: 281–319. doi:10.1007/978-94-017-9211-0_16.

Targeting p53-MDM2-MDMX Loop for Cancer Therapy

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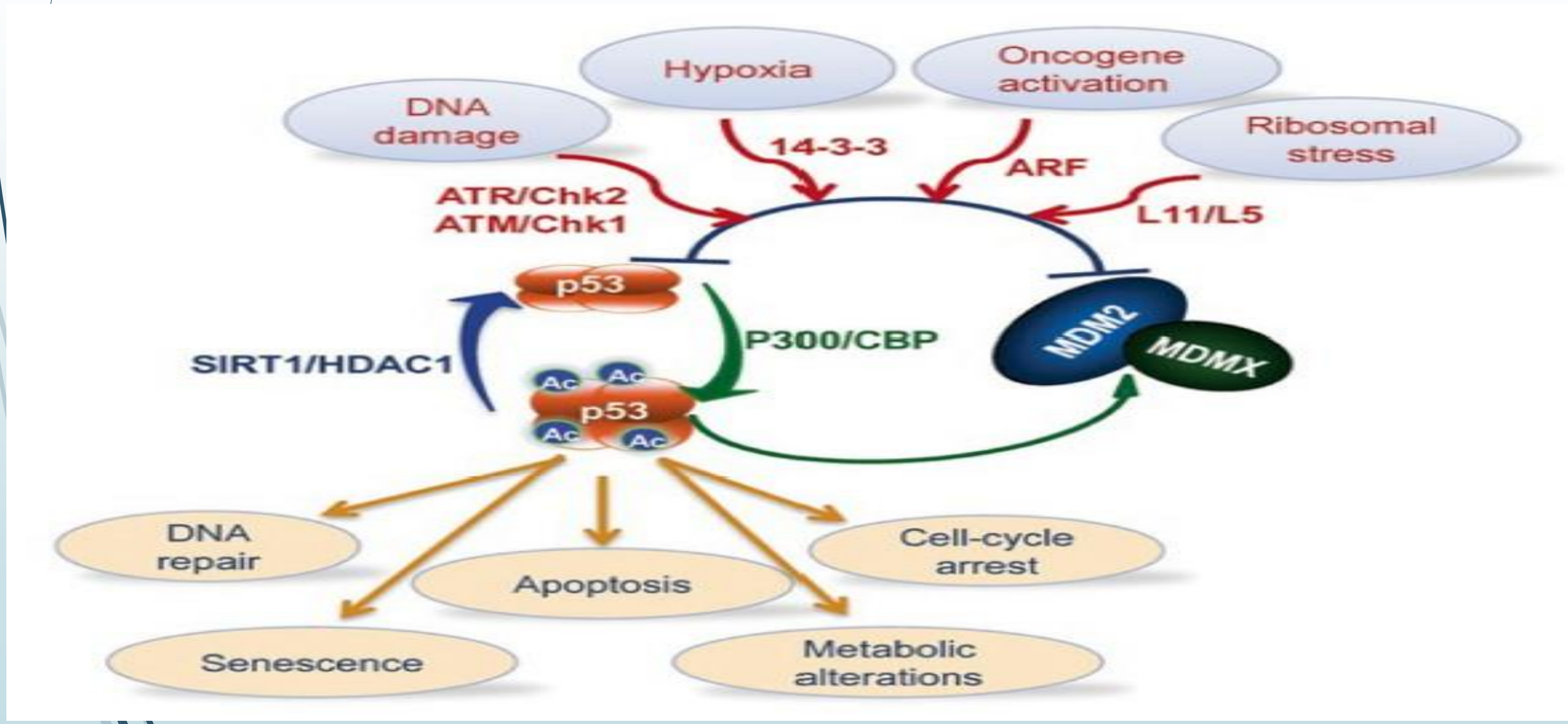
Hua Lu: hlu2@tulane.edu

Abstract

The tumor suppressor p53 plays a central role in anti-tumorigenesis and cancer therapy. It has been described as "the guardian of the genome", because it is essential for conserving genomic stability by preventing mutation, and its mutation and inactivation are highly related to all human cancers. Two important p53 regulators, MDM2 and MDMX, inactivate p53 by directly inhibiting its transcriptional activity and mediating its ubiquitination in a feedback fashion, as their genes are also the transcriptional targets of p53. On account of the importance of the p53-MDM2- MDMX loop in the initiation and development of wild type p53-containing tumors, intensive studies over the past decade have been aiming to identify small molecules or peptides that could specifically target individual protein molecules of this pathway for developing better anti-cancer therapeutics. In this chapter, we review the approaches for screening and discovering efficient and selective MDM2 inhibitors with emphasis on the most advanced synthetic small molecules that interfere with the p53-MDM2 interaction and are currently on Phase I clinical trials. Other therapeutically useful strategies targeting this loop, which potentially improve the prospects of cancer therapy and prevention, will also be discussed briefly.

The p53-MDM2-MDMX feedback loop(1)



Targeting p53-MDM2-MDMX Loop for Cancer Therapy(1)

- strategies to identify and discover MDM2/MDMX-targeted inhibitors

In Vitro Autoubiquitination and MDM2-Catalyzed p53 Ubiquitination Assay

Fluorescence-Based Biosensor High Content Screening Assay

affinity-based assays for high throughput screenings

NMR-based and isothermal calorimetric approach

Cell-Based Auto-ubiquitination Assay

Screening of Phage Display Library

Affinity-based assays for high throughput screenings(1)

- surface plasmon resonance(SPR)
- fluorescence polarization(FP)
- fluorescence resonance energy transfer(FRET)
- fluorescence-correlation spectroscopy experiments(FCS)

Targeting>>>synthetic molecules

MOLECULAR ONCOLOGY 2 (2008) 70–80

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

Mutant p53 targeting by the low molecular weight compound STIMA-1

Nicole Zache[a], *Jeremy M.R. Lambert*[a,c], *Nina Rökaeus*[a], *Jinfeng Shen*[a], *Pierre Hainaut*[c], *Jan Bergman*[b], *Klas G. Wiman*[a,*], *Vladimir J.N. Bykov*[a]

[a]*Karolinska Institutet, Department of Oncology-Pathology, Cancer Center Karolinska (CCK), Karolinska University Hospital, Stockholm, Sweden*

[b]*Karolinska Institutet, Department of Biosciences, Novum, Huddinge, Sweden*

[c]*International Agency for Research on Cancer (IARC), World Health Organization, Lyon, France*

Targeting>>>synthetic molecules

ABSTRACT

Reactivation of mutant p53 in human tumor cells should induce cell death by apoptosis and thus eliminate the tumor. Several small molecules that reactivate mutant p53 have been identified. Here we show that STIMA-1, a low molecular weight compound with some structural similarities to the previously identified molecule CP-31398, can stimulate mutant p53 DNA binding *in vitro* and induce expression of p53 target proteins and trigger apoptosis in mutant p53-expressing human tumor cells. Human diploid fibroblasts are significantly more resistant to STIMA-1 than mutant or wild type p53-carrying tumor cells. STIMA-1 may provide new insights into possible mechanisms of mutant p53 reactivation and thus facilitate the development of novel anticancer drugs that target mutant p53-carrying tumors.

Targeting>>>synthetic molecules

[Cell Cycle 6:16, 1995-2000, 15 August 2007]; ©2007 Landes Bioscience

Review

Targeting the p53 Family for Cancer Therapy

'Big Brother' Joins the Fight

Helen S. Bell

Kevin M. Ryan*

Tumor Cell Death Laboratory; Beatson Institute for Cancer Research; Cancer Research UK Beatson Laboratories; Glasgow UK

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http://www.landesbioscience.com/journals/cc/article/4614

KEY WORDS

p53, p73, iASPP, cell death, cancer, therapy

ABSTRACT

Inactivation of p53-mediated signaling plays a major role in both the genesis and therapy resistance of human cancer. Nearly all tumors contain mutations in p53 itself or have perturbations in the p53 pathway. Since there is clear evidence that many tumor cells are more likely to die in response to wild-type p53 activation or restoration than are their normal counterparts, there has been considerable interest in the development of small molecules that target p53 for therapeutic gain. These include compounds that either revert mutant p53 back to its wild-type conformation or compounds which interfere with the binding to, or the ubiquitylation of, p53 by MDM2. In both cases, however, the efficacy of the strategy depends on the presence of either mutant or wild-type p53 respectively thereby limiting their application to specific tumor settings. As a result, recent strategies have turned to the p53 family member, p73, which like p53 is a potent inducer of death, but in contrast is rarely lost or mutated in tumors. We discuss here all these different strategies and in particular focus on the discovery of an apoptotic peptide which targets not just p73, but potentially all p53 family members to cause tumor cell death.

Targeting>>>synthetic molecules

Cell Death and Differentiation (2006) 13, 921–926

www.nature.com/cdd

News and Commentary

Strategies for therapeutic targeting of the p53 pathway in cancer

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Cell Death and Differentiation (2006) **13**, 921–926.
doi:10.1038/sj.cdd.4401921; published online 24 March 2006

Targeting>>>synthetic molecules

Cell Death and Differentiation (2006) **13,** 921–926.
doi:10.1038/sj.cdd.4401921; published online 24 March 2006

The *TP53* gene is inactivated by point mutation in a large fraction of human tumors. p53 function is disrupted by indirect mechanisms in many wild-type *TP53*-carrying tumors as well. Loss of wild type p53 function allows apoptosis evasion and further selection of more malignant variants during tumor progression. Mutant *TP53*-carrying tumors show increased resistance to commonly used chemotherapeutic agents and radiotherapy. Therefore, p53 is an appealing target for novel anticancer therapeutic strategies. Several strategies for reactivation of the p53 pathway have been designed and tested during the last few years, such as *TP53* gene therapy and small molecules that reactivate mutant p53 or prevent Mdm2-mediated degradation of wild-type p53. Restoration of the p53 tumor suppressor pathway should trigger massive apoptosis, allowing rapid elimination of the tumor. The growing number of p53-targeting strategies raises hope for more efficient cancer therapy in the future.

The p53 pathway is almost certainly dysfunctional also in a majority of wild-type *TP53*-carrying tumors. This could occur through for example overexpression of the p53 antagonist Mdm2, loss of the Mdm2 inhibitor $p14^{ARF}$ via homozygous deletion of the *INK4a* locus, or expression of the human papilloma virus E6 protein that triggers p53 degradation (reviewed in Asker *et al.*[5]). It is clear, therefore, that reactivation of p53-induced apoptosis is a plausible and important therapeutic goal in many tumors regardless of *TP53* status.

Clinical studies have provided compelling evidence to support the notion that *TP53* mutations are associated with poor prognosis (see Olivier *et al.*[6] and the review by Royds and Iacopetta in this issue). This has been particularly well studied in breast and colon cancer. Mutations that reside in the L2 and L3 loops in the core domain and affect Zn binding and direct DNA contacts seem to be associated with the worst prognosis. This illustrates the fact that prognosis depends not only on the presence or absence of *TP53* mutation, but also on the exact localization of the mutation and the amino-acid substitution. The frequent p53 mutations in tumors and the fact that *TP53* mutation increases resistance to currently used radiotherapy and chemotherapy makes p53 and the p53 pathway an appealing target for novel cancer therapeutic strategies. Restoration of the p53 pathway should induce massive apoptosis and rapidly eliminate the tumor. Over the past few years, several exciting novel approaches for either activating p53 in wild-type *TP53*-carrying tumors or restoration of wild-type p53 function in mutant *TP53*-carrying tumors have been presented.

investigations on P53 gene therapy

Type of gene therapy	Type of targeting	Year	Related articles
targeting	RNA	2016	Targeted p53 activation by saRNA suppresses human bladder cancer cells growth and metastasis
		2016	Overexpression of p53 activated by small activating RNA suppresses the growth of human prostate cancer cells
		2014	Overexpression of MicroRNA-30b Improves Adenovirus-Mediated p53 Cancer Gene Therapy for Laryngeal Carcinoma
		2003	Gene silencing by adenovirus-delivered siRNA

Targeting>>>RNA

Wang et al. Journal of Experimental & Clinical Cancer Research (2016) 35:53
DOI 10.1186/s13046-016-0329-8

Targeted p53 activation by saRNA suppresses human bladder cancer cells growth and metastasis

Chenghe Wang[1,2†], Qiangqiang Ge[1†], Qingsong Zhang[1], Zhong Chen[1*], Jia Hu[1], Fan Li[1] and Zhangqun Ye[1]

Abstract

Background: Previous study showed that dsP53-285 has the capacity to induce tumor suppressor gene p53 expression by targeting promoter in non-human primates' cells. And it is well known that TP53 gene is frequently mutant or inactivated in human bladder cancer. Hereby, whether this small RNA can activate the expression of wild-type p53 and inhibit human bladder cancer cells remains to be elucidated.

Methods: Oligonucleotide and lentivirus were used to overexpress dsP53-285 and dsControl. Real-time PCR and western blot were used to detect genes' mRNA and protein expression, respectively. Cell proliferation assay, colony formation, flow cytometry, transwell assay and wound healing assay were performed to determine the effects on bladder cancer cells proliferation and migration/invasion *in vitro*. Animal models were carried out to analyze the effects on cells growth and metastasis *in vivo*.

Results: Transfection of dsP53-285 into human bladder cancer cell lines T24 and EJ readily activate wild-type p53 expression by targeting promoter. Moreover, dsP53-285 exhibited robust capacity to inhibit cells proliferation and colony formation, induce cells G0/G1 arrest, suppress migration and invasion. Besides, the Cyclin-CDK genes (Cyclin D1 and CDK4/6) were down-regulated and the EMT-associated genes (E-cadherin, β-catenin, ZEB1 and Vimentin) were also expressed inversely after dsP53-285 treatment. In addition, dsP53-285 could also significantly suppress the growth of bladder cancer xenografts and metastasis in nude mice. Most importantly, the anti-tumor effects mediated by dsP53-285 were mainly achieved by manipulating wild-type p53 expression.

Conclusion: Our findings indicate that the dsP53-285 can upregulate wild-type p53 expression in human bladder cancer cells through RNA activation, and suppresses cells proliferation and metastasis *in vitro* and *in vivo*.

Keywords: RNA activation, saRNA, Bladder cancer, Proliferation, Metastasis

Targeting>>>RNA

Overexpression of p53 activated by small activating RNA suppresses the growth of human prostate cancer cells

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8 January 2016
Number of times this article has been viewed

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*These authors contributed equally to this work

Abstract: Previous research has reported that a particular double-stranded RNA, named dsP53-285, has the capacity to induce expression of the tumor suppressor gene *TP53* in chimpanzee cells by targeting its promoter. Usually, it is the wild-type p53 protein, rather than mutants, which exhibits potent cancer-inhibiting effects. In addition, nonhuman primates, such as chimpanzees, share almost identical genome sequences with humans. This prompted us to speculate whether dsP53-285 can trigger wild-type p53 protein expression in human prostate cancer (PCa) cells and consequently suppress cell growth. The human PCa cell lines LNCaP and DU145 were transfected with dsP53-285 for 72 hours. Compared with the dsControl and mock transfection groups, expression of both p53 messenger RNA and p53 protein was significantly enhanced after dsP53-285 transfection, and this enhancement was followed by upregulation of p21, which indirectly indicated that dsP53-285 induced wild-type p53 expression. Moreover, overexpression of wild-type p53 mediated by dsP53-285 downregulated the expression of Cyclin D1 and cyclin-dependent kinase 4/6, thereby inducing PCa cell cycle arrest in G0/G1 phase and then inhibiting cell proliferation and clonogenicity. More importantly, dsP53-285 suppressed PCa cells mainly by modulating wild-type p53 expression. In conclusion, our study provides evidence that dsP53-285 can significantly stimulate wild-type p53 expression in the human PCa cell lines LNCaP and DU145 and can exert potent antitumor effects.

Keywords: p53, small activating RNA, prostate cancer

Targeting>>>RNA

Int. J. Mol. Sci. **2014**, *15*, 19729-19740; doi:10.3390/ijms151119729

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International Journal of
Molecular Sciences
ISSN 1422-0067
www.mdpi.com/journal/ijms

Article

Overexpression of MicroRNA-30b Improves Adenovirus-Mediated *p53* Cancer Gene Therapy for Laryngeal Carcinoma

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4 Department of Otolaryngology, Head and Neck Surgery, the Second Affiliated Hospital of Nanjing Medical University, 121 Jiangjiayuan Road, Nanjing 210011, China

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External Editor: Bing Yan

Targeting>>>RNA

Abstract: MicroRNAs play important roles in laryngeal carcinoma and other cancers. However, the expression of microRNAs in paracancerous tissue has been studied less. Here, using laser capture microdissection (LCM), we detected the expression of microRNAs in paracancerous tissues. Among all down-regulated microRNAs in the center area of tumor tissues, only miR-30b expression was significantly reduced in paracancerous tissues compared to surgical margins. Therefore, to further investigate the effect of miR-30b on laryngeal carcinoma, we stably overexpressed miR-30b in laryngeal carcinoma cell line HEp-2 cells. It was found that although there was no significant difference in cell viability between miR-30b overexpressed cells and control HEp-2 cells, *p53* expression was obviously enhanced in miR-30b overexpressed cells. Whether miR-30b could improve the anti-tumor effect of adenovirus-*p53* (Ad-*p53*) in laryngeal carcinoma and other cancer cell lines was also evaluated. It was found that in miR-30b overexpressed HEp-2 cells, p53-mediated tumor cell apoptosis was obviously increased both *in vitro* and *in vivo*. MDM2-p53 interaction might be involved in miR-30b-mediated

anti-tumor effect. Together, results suggested that miR-30b could modulate p53 pathway and enhance *p53* gene therapy-induced apoptosis in laryngeal carcinoma, which could provide a novel microRNA target in tumor therapy.

Keywords: laryngeal carcinoma; miR-30b; *p53*; field cancerization

Targeting>>>RNA

FEBS Letters 539 (2003) 111–114

Gene silencing by adenovirus-delivered siRNA

Changxian Shen[a], Andreas K. Buck[a], Xiangwei Liu[a], Michael Winkler[b], Sven N. Reske[a,*]

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[b] *Department of Virology, University of Ulm, D-89070 Ulm, Germany*

Received 10 January 2003; revised 2 February 2003; accepted 18 February 2003

First published online 5 March 2003

Edited by Varda Rotter

Abstract **RNA interference is the process that double-stranded RNA induces the homology-dependent degradation of cognate mRNA mediated by 21–23 nucleotide short interfering RNA (siRNA). Here, we describe a simple virus vector for efficient delivery of siRNA into mammalian cells utilizing the well-defined H1-RNA promoter and conventional adenovirus. In this pilot study, p53 was targeted by this vector. Our results demonstrate efficient and specific knock-down of p53 in breast cancer MCF-7 and lung carcinoma A549 cells and indicate a prospective application of this siRNA expressing recombinant adenovirus system in functional genomics, cancer gene therapy and virus inhibition.**

Key words: RNA interference; Short interfering RNA; p53; Adenovirus; Functional genomics

moter was also developed and demonstrated to mediate gene silencing both in vitro and in vivo [12]. With the completion of whole-genome sequencing of several organisms and extensive studies of functional genomics and proteomics, more and more genes will be validated for gene therapy. To expand the strategy of RNAi to human cancer gene therapy, we developed a simple adenovirus system utilizing the well defined polymerase III H1-RNA promoter to drive efficient expression of siRNA in mammalian cells. Our results demonstrate efficient and specific knock-down of p53 in different cell lines and indicate a promising application of this adenovirus system in functional genomics, cancer gene therapy and virus inhibition.

2. Materials and methods

investigations on P53 gene therapy

Type of gene therapy	Type of targeting	year	Related articles
targeting	Crispr/ cas	2017	Repurposing CRISPR/Cas9 for in situ functional assays
		2016	CRISPR-Cas9–based target validation for p53-reactivating model compounds
		2015	Somatic CRISPR/Cas9-mediated tumour suppressor disruption enables versatile brain tumour modelling
		2014	CRISPR-mediated direct mutation of cancer genes in the mouse liver

Targeting>>>Crispr/cas

Repurposing CRISPR/Cas9 for in situ functional assays

Abba Malina,[1,6] John R. Mills,[1,6,7] Regina Cencic,[1] Yifei Yan,[2] James Fraser,[1] Laura M. Schippers,[1] Marilène Paquet,[3] Josée Dostie,[1] and Jerry Pelletier[1,4,5,8]

[1]Department of Biochemistry, McGill University, Montreal, Quebec H3G 1Y6 Canada,; [2]Département de Biochimie et Médecine Moléculaire, Université de Montréal, Quebec H3C 3J7, Canada; [3]Département de Pathologie et de Microbiologie, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec J2S 2M2, Canada; [4]Department of Oncology, McGill University, Montreal, Quebec H3G 1Y6, Canada; [5]The Rosalind and Morris Goodman Cancer Research Center, McGill University, Montreal, Quebec H3G 1Y6, Canada

RNAi combined with next-generation sequencing has proven to be a powerful and cost-effective genetic screening platform in mammalian cells. Still, this technology has its limitations and is incompatible with in situ mutagenesis screens on a genome-wide scale. Using p53 as a proof-of-principle target, we readapted the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR associated 9) genome-editing system to demonstrate the feasibility of this methodology for targeted gene disruption positive selection assays. By using novel "all-in-one" lentiviral and retroviral delivery vectors heterologously expressing both a codon-optimized Cas9 and its synthetic guide RNA (sgRNA), we show robust selection for the CRISPR-modified *Trp53* locus following drug treatment. Furthermore, by linking Cas9 expression to GFP fluorescence, we use an "all-in-one" system to track disrupted *Trp53* in chemoresistant lymphomas in the Eμ-*myc* mouse model. Deep sequencing analysis of the tumor-derived endogenous Cas9-modified *Trp53* locus revealed a wide spectrum of mutants that were enriched with seemingly limited off-target effects. Taken together, these results establish Cas9 genome editing as a powerful and practical approach for positive in situ genetic screens.

[*Keywords*: Cas9; CRISPR; genome editing; functional screening; p53]

Targeting>>>Crispr/cas

NATURE CHEMICAL BIOLOGY | ARTICL

CRISPR-Cas9–based target validation for p53-reactivating model compounds

- Michael Wanzel ,Jonas B Vischedyk ,Miriam P Gittler ,Niklas Gremke
- Julia R Seiz ,Miriam Hefter ,Magdalena Noack ,Raikumar Savai
- Marco Mernberger ,Joël P Charles ,Jean Schneikert ,Anne Catherine Bretz
- Andrea Nist ,Thorsten Stiewe

Nature Chemical Biology

Abstract

Inactivation of the p53 tumor suppressor by Mdm2 is one of the most frequent events in cancer, so compounds targeting the p53-Mdm2 interaction are promising for cancer therapy. Mechanisms conferring resistance to p53-reactivating compounds are largely unknown. Here we show using CRISPR-Cas9–based target validation in lung and colorectal cancer that the activity of nutlin, which blocks the p53-binding pocket of Mdm2, strictly depends on functional p53. In contrast, sensitivity to the drug RITA, which binds the Mdm2-interacting N terminus of p53, correlates with induction of DNA damage. Cells with primary or acquired RITA resistance display cross-resistance to DNA crosslinking compounds such as cisplatin and show increased DNA cross-link repair. Inhibition of FancD2 by RNA interference or pharmacological mTOR inhibitors restores RITA sensitivity. The therapeutic response to p53-reactivating compounds is therefore limited by compound-specific resistance mechanisms that can be resolved by CRISPR-Cas9–based target validation and should be considered when allocating patients to p53-reactivating treatments.

Targeting>>>Crispr/cas

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DOI: 10.1038/ncomms8391

nature COMMUNICATIONS

ARTICLE

Received 28 Jan 2015 | Accepted 1 May 2015 | Published 11 Jun 2015

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Somatic CRISPR/Cas9-mediated tumour suppressor disruption enables versatile brain tumour modelling

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In vivo functional investigation of oncogenes using somatic gene transfer has been successfully exploited to validate their role in tumorigenesis. For tumour suppressor genes this has proven more challenging due to technical aspects. To provide a flexible and effective method for investigating somatic loss-of-function alterations and their influence on tumorigenesis, we have established CRISPR/Cas9-mediated somatic gene disruption, allowing for *in vivo* targeting of TSGs. Here we demonstrate the utility of this approach by deleting single (*Ptch1*) or multiple genes (*Trp53, Pten, Nf1*) in the mouse brain, resulting in the development of medulloblastoma and glioblastoma, respectively. Using whole-genome sequencing (WGS) we characterized the medulloblastoma-driving *Ptch1* deletions in detail and show that no off-targets were detected in these tumours. This method provides a fast and convenient system for validating the emerging wealth of novel candidate tumour suppressor genes and the generation of faithful animal models of human cancer.

Targeting>>>Crispr/cas

NATURE | LETTER

CRISPR-mediated direct mutation of cancer genes in the mouse liver

Wen Xue, Sidi Chen, Hao Yin, Tuomas Tammela, Thales Papagiannakopoulos, Nikhil S. Joshi, Wenxin Cai, Gillian Yang, Roderick Bronson, Denise G. Crowley, Feng Zhang, Daniel G. Anderson, Phillip A. Sharp & Tyler Jacks

Nature

Abstract

The study of cancer genes in mouse models has traditionally relied on genetically-engineered strains made via transgenesis or gene targeting in embryonic stem cells[1]. Here we describe a new method of cancer model generation using the CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins) system *in vivo* in wild-type mice. We used hydrodynamic injection to deliver a CRISPR plasmid DNA expressing Cas9 and single guide RNAs (sgRNAs)[2,3,4] to the liver that directly target the tumour suppressor genes *Pten* (ref. 5) and *p53* (also known as *TP53* and *Trp53*) (ref. 6), alone and in combination. CRISPR-mediated *Pten* mutation led to elevated Akt phosphorylation and lipid accumulation in hepatocytes, phenocopying the effects of deletion of the gene using Cre–*LoxP* technology[7,8]. Simultaneous targeting of *Pten* and *p53* induced liver tumours that mimicked those caused by Cre–*loxP*-mediated deletion of *Pten* and *p53*. DNA sequencing of liver and tumour tissue revealed insertion or deletion mutations of the tumour suppressor genes, including bi-allelic mutations of both *Pten* and *p53* in tumours. Furthermore, co-injection of Cas9 plasmids harbouring sgRNAs targeting the β-catenin gene and a single-stranded DNA oligonucleotide donor carrying activating point mutations led to the generation of hepatocytes with nuclear localization of β-catenin. This study demonstrates the feasibility of direct mutation of tumour suppressor genes and oncogenes in the liver using the CRISPR/Cas system, which presents a new avenue for rapid development of liver cancer models and functional genomics

investigations on P53 gene therapy

Type of gene therapy	year	Related articles
exosome	2014	Interactions between Exosomes from Breast Cancer Cells and Primary Mammary Epithelial Cells Leads to Generation of Reactive Oxygen Species Which Induce DNA Damage Response, Stabilization of p53 and Autophagy in Epithelial Cells
	2008	Exosome secretion, including the DNA damage-induced p53-dependent secretory pathway, is severely compromised in TSAP6/Steap3-null mice

exosome

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Interactions between Exosomes from Breast Cancer Cells and Primary Mammary Epithelial Cells Leads to Generation of Reactive Oxygen Species Which Induce DNA Damage Response, Stabilization of p53 and Autophagy in Epithelial Cells

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Abstract

Exosomes are nanovesicles originating from multivesicular bodies and are released by all cell types. They contain proteins, lipids, microRNAs, mRNAs and DNA fragments, which act as mediators of intercellular communications by inducing phenotypic changes in recipient cells. Tumor-derived exosomes have been shown to play critical roles in different stages of tumor development and metastasis of almost all types of cancer. One of the ways by which exosomes affect tumorigenesis is to manipulate the tumor microenvironments to create tumor permissive "niches". Whether breast cancer cell secreted exosomes manipulate epithelial cells of the mammary duct to facilitate tumor development is not known. To address whether and how breast cancer cell secreted exosomes manipulate ductal epithelial cells we studied the interactions between exosomes isolated from conditioned media of 3 different breast cancer cell lines (MDA-MB-231, T47DA18 and MCF7), representing three different types of breast carcinomas, and normal human primary mammary epithelial cells (HMECs). Our studies show that exosomes released by breast cancer cell lines are taken up by HMECs, resulting in the induction of reactive oxygen species (ROS) and autophagy. Inhibition of ROS by N-acetyl-L-cysteine (NAC) led to abrogation of autophagy. HMEC-exosome interactions also induced the phosphorylation of ATM, H2AX and Chk1 indicating the induction of DNA damage repair (DDR) responses. Under these conditions, phosphorylation of p53 at serine 15 was also observed. Both DDR responses and phosphorylation of p53 induced by HMEC-exosome interactions were also inhibited by NAC. Furthermore, exosome induced autophagic HMECs were found to release breast cancer cell growth promoting factors. Taken together, our results suggest novel mechanisms by which breast cancer cell secreted exosomes manipulate HMECs to create a tumor permissive microenvironment.

exosome

ell Death Differ. 2008 Nov;15(11):1723-33. doi: 10.1038/cdd.2008.104. Epub 2008 Jul 11.

Exosome secretion, including the DNA damage-induced p53-dependent secretory pathway, is severely compromised in TSAP6/Steap3-null mice.

Lespagnol A[1], Duflaut D, Beekman C, Blanc L, Fiucci G, Marine JC, Vidal M, Amson R, Telerman A.
Author information

Abstract

TSAP6 (tumor suppressor-activated pathway 6), also known as Steap3, is a direct p53 transcriptional target gene. It regulates protein secretion, for example translationally controlled tumor protein (TCTP), which is implicated in tumor reversion. In keeping with the latter, we show herein that TSAP6 is a glycosylated protein present in the trans-Golgi network, endosomal-vesicular compartment and cytoplasmic membrane. To further investigate the physiological function of TSAP6, we have generated TSAP6-deficient mice. These mice exhibit microcytic anemia with abnormal reticulocyte maturation and deficient transferrin receptor downregulation, a process known to be dependent on exosomal secretion. Moreover, we provide direct evidence that exosome production is severely compromised in TSAP6-null cells. Finally, we show that the DNA damage-induced p53-dependent nonclassical exosomal secretory pathway is abrogated in TSAP6-null cells. Given the fact that exosomes are used as cell-free vaccines against cancer and that they could be involved in the biogenesis and spread of human immunodeficiency virus, it is important to understand their regulation. The results presented here provide the first genetic demonstration that exosome formation is a tightly controlled biological process dependent of TSAP6

investigations on P53 gene therapy

Type of gene therapy	Additional therapeutic	year	Related articles
P53 combination with other therapeutic	radiotherapy	2015	A patient with a large intrathoracic malignant schwannoma who showed a complete clinical response to rAd-p53-combined with radiotherapy.
	ING4	2015	Adenovirus-mediated p53 and ING4 gene co-transfer elicits synergistic antitumor effects through enhancement of p53 acetylation in breast cancer
	radiotherapy and hypoxia	2015	p53 activated by AND gate genetic circuit under radiation and hypoxia for targeted cancer gene therapy
	LAK	2014	A combined lymphokine-activated killer (LAK) cell immunotherapy and adenovirus-p53 gene therapy for head and neck squamous cell carcinoma

P53 combination with other therapeutic (Radiotherapy)

Anticancer Drugs. 2015 Sep;26(8):902-6. doi: 10.1097/CAD.0000000000000264.

A patient with a large intrathoracic malignant schwannoma who showed a complete clinical response to rAd-p53-combined with radiotherapy.

Liu K[1], Zhao J, Jiang H, Ma J, Tan J, Pei Y, Chen J.
Author information

Abstract

The prognosis of postoperatively recurred malignant schwannoma is poor and there is no effective treatment. We had a patient who was found to have a large intrathoracic tumor 1 year after surgery and could not tolerate an operation for the second time. We then decided to evaluate the synergistic effect of recombinant adenovirus-p53 (rAd-p53) combined with radiotherapy for the patient. rAd-p53 was injected intratumorally twice a week before radiotherapy, a total of 10 times, over a course of treatment. Radiotherapy then followed gene therapy at five fractions a week for 5 weeks, with a total dosage of 80.6 Gy/31f in the center part of the tumor and 62 Gy/31f in other locations. The pathological diagnosis of malignant schwannoma indicated that the p53 expression was strongly positive and vascular endothelial growth factor and Bcl-2 were positive before treatment on protein immunohistochemical staining. After treatment, the diameter of the tumor was noticeably reduced and the center part of the tumor presented as a fluid anechoic area and cavities on computed tomographic scanning. The result of the puncture biopsy showed that there were many fibronecrotic tissues and no significant tumor cells. The p53 expression was weakly positive, Vascular endothelial growth factor was negative, and Bcl-2 was weakly positive after treatment on protein immunohistochemical staining

P53 combination with other therapeutic (ING)

Oncol Rep. 2016 Jan;35(1):243-52. doi: 10.3892/or.2015.4385. Epub 2015 Nov 3.

Adenovirus-mediated p53 and ING4 gene co-transfer elicits synergistic antitumor effects through enhancement of p53 acetylation in breast cancer.

Wu J[1], Zhu Y[1], Xu C[1], Xu H[1], Zhou X[1], Yang J[2], Xie Y[1], Tao M[1].

Abstract

Multigene-based combination therapy may be an effective practice in cancer gene therapy. Substantial studies have demonstrated that tumor suppressor p53 acetylation is indispensable for p53 activation. Inhibitor of growth 4 (ING4), as a novel tumor suppressor, is capable of remarkably enhancing p53 acetylation and its transcriptional activity. Hence, we assumed that combined treatment of p53 and ING4 double tumor suppressors would exhibit enhanced antitumor effects. The combined therapeutic efficacy of p53 and ING4 for human cancers has not been previously reported. We thus generated multiple promoter expression cassette-based recombinant adenovirus-co-expressing ING4 and p53 double tumor suppressor genes (AdVING4/p53), evaluated the combined effects of AdVING4/p53 on breast cancer using the MDA-MB-231 (mutant p53) human breast cancer cell line, and also elucidated its underlying molecular mechanisms. We demonstrated that AdVING4/p53-mediated p53 and ING4 co-expression induced synergistic growth inhibition and apoptosis as well as enhanced effects on upregulation of acetylated p53, P21, Bax, PUMA, Noxa, cleaved caspase-9, cleaved caspase-3 and cleaved PARP, and downregulation of Bcl-2, CD31 and microvessel density (MVD) in MDA-MB-231 breastcancer in vitro and/or in vivo subcutaneous (s.c.) xenografted tumors. The synergistic antitumor activity elicited by AdVING4/p53 was closely associated with the enhanced activation of the intrinsic apoptotic pathway and synergistic inhibition of tumor angiogenesis, very possibly via ING4-mediated enhancement of p53 acetylation and activity. Thus, our results indicate that cancer gene therapy combining two or more tumor suppressors such as p53 and ING4 may constitute a novel and effective therapeutic modality for human breast cancer and other cancers

P53 combination with other therapeutic (AND)

Cancer Sci **106** (2015) 1163–1173

doi: 10.1111/cas.12739

Cancer Science

Japanese Cancer Association JCA

Open Access

p53 activated by AND gate genetic circuit under radiation and hypoxia for targeted cancer gene therapy

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Key words

AND gate, cancer, hypoxia, radiation, targeted gene therapy

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Cancer Sci **106** (2015) 1163–1173

Radio-activated gene therapy has been developed as a novel therapeutic strategy against cancer; however, expression of therapeutic gene in peritumoral tissues will result in unacceptable toxicity to normal cells. To restrict gene expression in targeted tumor mass, we used hypoxia and radiation tolerance features of tumor cells to develop a synthetic AND gate genetic circuit through connecting radiation sensitivity promoter $cArG_6$, heat shock response elements SNF1, HSF1 and HSE_4 with retroviral vector plxsn. Their construction and dynamic activity process were identified through downstream enhanced green fluorescent protein and wtp53 expression in non-small cell lung cancer A549 cells and in a nude mice model. The result showed that AND gate genetic circuit could be activated by lower required radiation dose (6 Gy) and after activated, AND gate could induce significant apoptosis effects and growth inhibition of cancer cells *in vitro* and *in vivo*. The radiation- and hypoxia-activated AND gate genetic circuit, which could lead to more powerful target tumoricidal activity represented a promising strategy for both targeted and effective gene therapy of human lung adenocarcinoma and low dose activation character of the AND gate genetic circuit implied that this model could be further exploited to decrease side-effects of clinical radiation therapy.

P53 combination with other therapeutic (LAK)

Anticancer Res. 2014 Jul;34(7):3365-70.

A combined lymphokine-activated killer (LAK) cell immunotherapy and adenovirus-p53 gene therapy for head and neck squamous cell carcinoma.

Saito H[1], Ando S[2], Morishita N[1], Lee KM[3], Dator D[4], Dy D[5], Shigemura K[6], Adhim Z[7], Nibu K[7], Fujisawa M[7], Shirakawa T[8].

Abstract

BACKGROUND:

The antitumor activity of lymphokine activated killer (LAK) cells immunotherapy is not always effective in all patients, especially when used alone. In this study, we investigated the in vitro antitumor activities of a combination of LAK immunotherapy and gene therapy employing an adenovirus carrying the p53 gene (Ad-p53) in human head and neck squamous cell carcinoma.

MATERIALS AND METHODS:

The in vitro cytotoxicity of LAK cells was tested in H891 cells infected with or without Ad-p53, and the mRNA expression levels of natural killer group 2D ligands (UL16 binding protein (ULBP) 1 to 5) and tumor necrosis factor (TNF-α) in these cells were measured by real-time reverse transcription polymerase chain reaction.

RESULTS:

Ad-p53 infection increased the cytotoxicity of LAK cells against H891 cells, and also increased the mRNA expression levels of the ULBPs in H891 cells and TNF-α in the LAK cells.

CONCLUSION:

The antitumor activities of LAK cells in H891 cells were enhanced by Ad-p53.

CONCLUSION:

The combinational therapy of LAK immunotherapy and Ad-p53 gene therapy may represent a new paradigm for the treatment of head and neck cancer

investigations on P53 gene therapy

Type of gene therapy	Additional therapeutic	Year	
P53 combination with other therapeutic	siRNA	2013	Plasmid-based E6-specific siRNA and co-expression of wild-type p53 suppresses the growth of cervical cancer in vitro and in vivo
	Penetrating peptide 11R	2013	Anticancer activity of oncolytic adenoviruses carrying p53 is augmented by 11R in gallbladder cancer cell lines in vitro and in vivo
	Ribosomal Protein L23	2013	Co-Transduction of Ribosomal Protein L23 Enhances the Therapeutic Efficacy of Adenoviral-Mediated P53 Gene Transfer in Human Gastric Cancer

P53 combination with other therapeutic (siRNA)

Published in final edited form as:
Cancer Lett. 2013 July 10; 335(1): 242–250. doi:10.1016/j.canlet.2013.02.034.

Plasmid-based E6-specific siRNA and co-expression of wild-type p53 suppresses the growth of cervical cancer in vitro and in vivo

Xin Li[a,d,1], **Yang Li**[a,d,1], **Jiadi Hu**[b,d], **Bo Wang**[a,d], **Lijing Zhao**[a,d], **Kun Ji**[a,d], **Baofeng Guo**[a,d], **Di Yin**[a,d], **Yanwei Du**[a,d], **Dennis J. Kopecko**[c,d], **Dhananjaya V. Kalvakolanud**[c,d], **Xuejian Zhao**[a,d], **Deqi Xu**[d,e,*], and **Ling Zhang**[a,d,*]

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[e]National New Vaccine Engineering Research Center, Beijing 100024, PR China

Abstract

The E6 protein of the oncogenic HPV-16 functions by interfering with the normal cell cycle control mechanisms, particularly those controlled by p53. In this study, we developed a dual expression plasmid that coexpressed-E6-specific siRNA and wild type p53, and to evaluate its effects on cervical cancer growth. We found that simultaneous expression of pSi-E6-P53 caused a robust suppression of tumor growth when compared to the controls either E6-specific siRNA or p53 alone. In conclusion, our findings demonstrate that a combined strategy of co-expressed E6-specific siRNA and p53 synergistically and more effectively suppressed cervical tumor growth when compared with single treatment.

P53 combination with other therapeutic (siRNA)

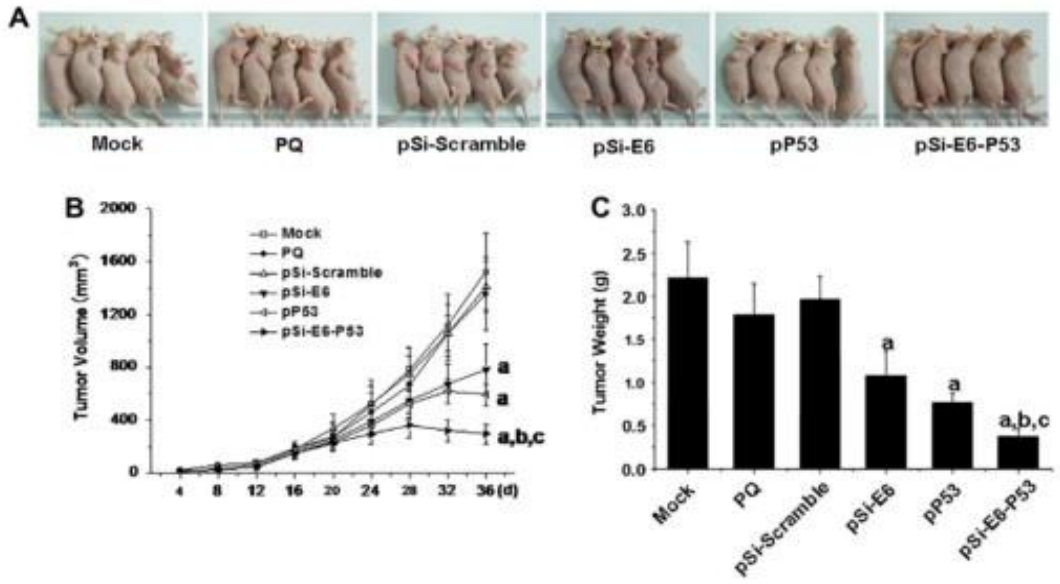

Fig. 4.
Inhibition of tumor growth in vivo by treatments with various plasmids. (A) Images of mice with different plasmid treatments collected after sacrifice at day 36. (B) Tumor growth cures over the 36-day period was established based on the tumor sizes measured every fourth day. (C) Tumor wet weights measured after sacrifice at day 36. The data were presented as the mean ± SD of triplicate experiments. a, $P < 0.05$ versus Mock, PQ, or pSi-Scramble group. b, $P < 0.05$ versus pSi-E6 group. c, $P < 0.05$ versus pP53 group.

P53 combination with other therapeutic (siRNA)

Mouse and tumor weights and tumor volume after treatments.

Group	Mean mouse weight (g)	Mean tumor weight (g)	Mean tumor volume (mm^3)
Mock	22.17 ± 2.00	2.21 ± 0.41	1525.46 ± 285.97
PQ	23.23 ± 1.21	1.78 ± 0.36	1356.28 ± 276.53
pSi-Scramble	21.87 ± 1.43	1.96 ± 0.27	1411.81 ± 193.93
pSi-E6	23.37 ± 1.79	1.07 ± 0.29[a]	784.50 ± 190.96[a]
pP53	22.61 ± 0.86	0.76 ± 0.11[a]	594.30 ± 83.40[a]
pSi-E6-P53	23.76 ± 1.48	0.37 ± 0.09[a,b,c]	296.07 ± 74.22[a,b,c]

The data are presented as means ± SD of triplicate experiments.

[a] $P < 0.05$ versus Mock, PQ, or pSi-Scramble group.

[b] $P < 0.05$ versus pSi-E6 group.

[c] $P < 0.05$ versus pP53 group.

P53 combination with other therapeutic (Penetrating peptide 11R)

Anticancer activity of oncolytic adenoviruses carrying p53 is augmented by 11R in gallbladder cancer cell lines *in vitro* and *in vivo*

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DOI: 10.3892/or.2013.2511

Abstract. Gallbladder cancer (GBC) is a rare disease associated with an extremely poor patient prognosis, and occasionally, aberrant expression of p53 is present. Considering that p53 is one of the most widely studied tumor-suppressor genes, we used a cell-penetrating peptide, 11R, to enhance the transferring efficiency of the oncolytic adenovirus carrying the p53 gene by constructing SG7605-11R-p53, a gene-viral therapy system which has higher specificity, enhanced safety, and efficacy. After infection with SG7605-11R-p53 at a multiplicity of infection (MOI) of 1 PFU/cell *in vitro*, the survival rate of EH-GB1 cells was lower than 50%, and that of EH-GB2 cells was lower than 40%, while the survival rate was higher than 90% for BJ human fibroblast cells, demonstrating that SG7605-11R-p53 has potent specific cytotoxicity against GBC cells. The tumor growth was greatly inhibited in nude mice bearing EH-GB2 xenografts when the total dose of SG7605-11R-p53 was $1x10^9$ PFU, and terminal dUTP nick end-labeling (TUNEL) revealed that the apoptotic rate of cancer cells was 66.75±6.702%. Compared with existing gene therapy with long-standing shortcomings, our new system offers an additional option for patients with advanced GBC and other cancers who may not be suitable for chemotherapy, radiotherapy or who are not indicated for surgical treatment.

P53 combination with other therapeutic (Ribosomal Protein L23)

Co-transduction of ribosomal protein L23 enhances the therapeutic efficacy of adenoviral-mediated p53 gene transfer in human gastric cancer

YA-FEI ZHANG[1,2*], BI-CHENG ZHANG[1*], AN-RAN ZHANG[2], TING-TING WU[1], JIAN LIU[1], LI-FANG YU[1], WEI-XING WANG[1], JIAN-FEI GAO[1], DIAN-CHUN FANG[2] and ZHI-GUO RAO[1]

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DOI: 10.3892/or.2013.2663

Abstract. Induction of murine double minute 2 (MDM2) expression is thought to be a determinant of resistance to p53 gene therapy for cancer. Previous studies have revealed that ribosomal protein L23 (RPL23) inhibits MDM2-mediated p53 degradation through direct binding to MDM2. In addition, ectopically expressed RPL23 was reported to interact with MDM2 in both the nucleus and cytoplasm, by which RPL23 indirectly inhibited MDM2-p53 binding. Based on the known molecular properties of the RPL23 protein, it was speculated that co-transduction of RPL23 may protect wild-type p53 protein from MDM2-mediated inactivation and, thus, improve the effect of delivering therapeutic exogenous p53. To test this hypothesis, we constructed a bicistronic adenoviral vector expressing both the RPL23 and p53 genes (Ad-RPL23/p53) and compared its tumor-suppressor activity in human gastric cancer with that of a single gene vector for p53 (Ad-p53). In the *in vivo* and *in vitro* experiments, we observed that treatment with Ad-RPL23/p53 resulted in a stronger antitumor response compared to that obtained using Ad-p53. Moreover, the antitumor response of the bicistronic adenovirus was obtained not only in MGC803 cells (endogenous mutant p53) were initially resistant to p53 gene transfer, indicating that co-transduction of RPL23 also expanded the utility of p53 gene therapy. Furthermore, in an orthotopic nude mouse model of human gastric cancer, we found that the survival benefit was greater after Ad-RPL23/p53 treatment than after Ad-p53. Taken together, the data presented here demonstrate that co-transduction of RPL23 enhances the therapeutic efficacy of adenoviral-mediated p53 gene transfer in models of human gastric cancer and support the use of this strategy for cancer treatment.

investigations on P53 gene therapy

Type of gene therapy	Type of vectors	Type of vectors	year	Related articles
Vectors	**Viral**	1)adeno virus mediated	2013	1) Recombinant human adenovirus-p53 injection induced apoptosis in hepatocellular carcinoma cell lines mediated by p53-Fbxw7 pathway, which controls c-Myc and cyclin E
			2013	2) . Advances in adenovirus-mediated p53 cancer gene therapy.
		2)Retro virus	1996	1) Retrovirus–mediated wild–type P53 gene transfer to tumors of patients with lung cancer
		3)sv40 virus	1994	1) Induction versus progression of brain tumor development: differential functions for the pRB- and p53-targeting domains of simian virus 40 T antigen
	Non viral	1)liposomes (lipoplexes) 2)Nano carier 3)dendrimers		Next slide

Viral vectors(adeno virus mediated)

OPEN ACCESS Freely available online

PLOS ONE

Recombinant Human Adenovirus-p53 Injection Induced Apoptosis in Hepatocellular Carcinoma Cell Lines Mediated by p53-Fbxw7 Pathway, Which Controls c-Myc and Cyclin E

Kangsheng Tu, Xin Zheng, Zhenyu Zhou, Chao Li, Jing Zhang, Jie Gao, Yingmin Yao, Qingguang Liu*

Department of Hepatobiliary Surgery, First Affiliated Hospital of Medical College of Xi'an Jiaotong University, Xi'an, Shaanxi, China

Abstract

F-box and WD repeat domain-containing 7 (Fbxw7/hAgo/hCdc4/Fbw7) is a p53-dependent tumor suppressor and leads to ubiquitination-mediated suppression of several oncoproteins including c-Myc, cyclin E, Notch, c-Jun and others. Our previous study has indicated that low expression of Fbxw7 was negatively correlated with c-Myc, cyclin E and mutant-p53 in hepatocellular carcinoma (HCC) tissues. But the role and mechanisms of Fbxw7 in HCC are still unknown. Here, we investigated the function of Fbxw7 in HCC cell lines and the anti-tumor activity of recombinant human adenovirus-p53 injection (rAd-p53, Gendicine) administration *in vitro* and *in vivo*. Fbxw7-specific siRNA enhanced expression of c-Myc and cyclin E proteins and increased proliferation in cell culture. rAd-p53 inhibited tumor cell growth with Fbxw7 upregulation and c-Myc and cyclin E downregulation *in vitro* and a murine HCC model. This effect could be partially reverted using Fbxw7-specific siRNA. Here, we suggest that the activation of Fbxw7 by adenoviral delivery of p53 leads to increased proteasomal degradation of c-Myc and cyclin E enabling growth arrest and apoptosis. Addressing this pathway, we identified that rAd-p53 could be a potential therapeutic agent for HCC.

Viral vectors(adeno virus mediated)

Expert Opin Biol Ther. 2013 Nov;13(11):1569-83. doi: 10.1517/14712598.2013.845662.

Advances in adenovirus-mediated p53 cancer gene therapy.

Tazawa H[1], Kagawa S, Fujiwara T.

Abstract

INTRODUCTION:

The tumor suppressor p53 gene regulates diverse cellular processes, such as cell-cycle arrest, senescence, apoptosis and autophagy, and it is frequently inactivated by genetic alterations in ~ 50% of all types of human cancers. To restore wild-type p53 function in p53-inactivated tumors, adenovirus-mediated p53 gene therapy has been developed as a promising antitumor strategy in preclinical experiments and clinical studies.

AREAS COVERED:

This review focuses on the clinical relevance of replication-deficient adenovirus vectors that carry the wild-type p53 gene (Ad-p53; Advexin, Gendicine and SCH-58500) in clinical studies of patients with various cancers and the future perspectives regarding conditionally replicating adenovirus vectors expressing the wild-type p53 gene (CRAd-p53; AdDelta24-p53, SG600-p53, OBP-702) in preclinical experiments. Moreover, the recent advances in our understanding of the molecular basis for the p53-mediated tumor suppression network induced by Ad-p53 and CRAd-p53 vectors and the combination therapies for promoting the therapeutic potential of adenovirus-mediated p53 gene therapy are discussed.

EXPERT OPINION:

Exploration of the molecular mechanism underlying the p53-mediated tumor suppression network and the effective strategy for enhancing the p53-mediated cell death signaling pathway would provide novel insights into the improvement of clinical outcome in p53-based cancer gene therapy

Viral vectors(Retrovirus)

Nature Medicine **2**, 985 - 991 (1996)
doi:10.1038/nm0996-985

Retrovirus–mediated wild–type *P53* gene transfer to tumors of patients with lung cancer.

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A retroviral vector containing the wild–type *p53* gene under control of a β–actin promoter was produced to mediate transfer of wild–type *p53* into human non–small cell lung cancers by direct injection. Nine patients whose conventional treatments failed were entered into the study. No clinically significant vector–related toxic effects were noted up to five months after treatment. *In situ* hybridization and DNA polymerase chain reaction showed vector–*p53* sequences in posttreatment biopsies. Apoptosis (programmed cell death) was more frequent in posttreatment biopsies than in pretreatment biopsies. Tumor regression was noted in three patients, and tumor growth stabilized in three other patient

Viral vectors(sv40 virus)

Induction versus progression of brain tumor development: differential functions for the pRB- and p53-targeting domains of simian virus 40 T antigen.

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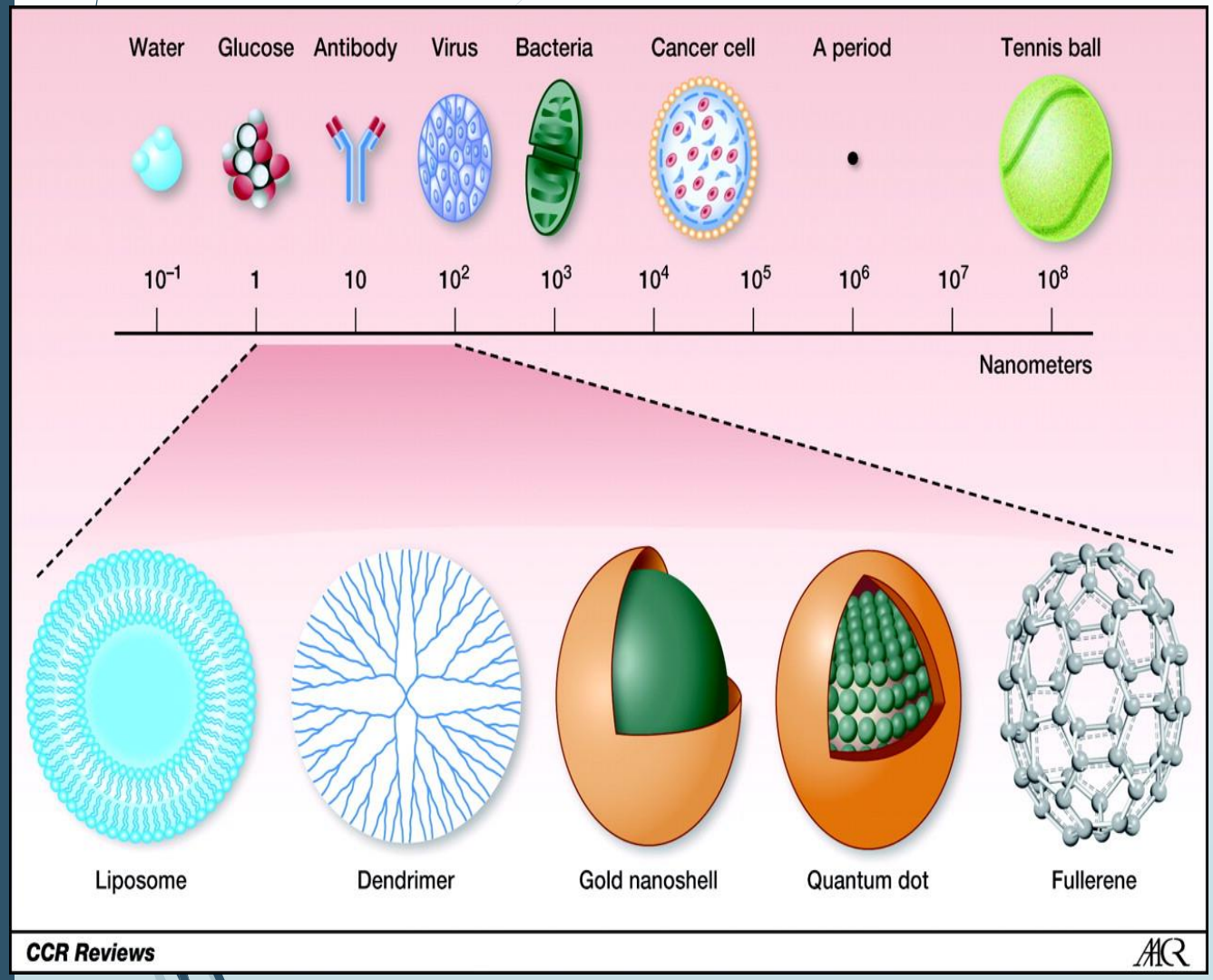
ABSTRACT

The ability of simian virus 40-encoded large T antigen to disrupt the growth control of a variety of cell types is related to its ability to interfere with certain cellular proteins, such as p53 and the retinoblastoma susceptibility gene product (pRB). We have used wild-type and mutant forms of T antigen in transgenic mice to dissect the roles of pRB, p53, and other cellular proteins in tumorigenesis of different cell types. In this study, using a cell-specific promoter to target expression specifically to brain epithelium (the choroid plexus) and to B and T lymphoid cells, we characterize the tumorigenic capacity of a T-antigen fragment that comprises only the amino-terminal 121 residues. This fragment (dl1137) retains the ability to interact with pRB and p107 but lacks the p53-binding domain. While loss of the p53-binding region results in loss of the capacity to induce lymphoid abnormalities, dl1137 retains the ability to induce choroid plexus tumors that are histologically indistinguishable from those induced by wild-type T antigen. Tumors induced by dl1137 develop much more slowly, however, reaching an end point at around 8 months of age rather than at 1 to 2 months. Analysis of tumor progression indicates that tumor induction by dl1137 does not require secondary genetic or epigenetic events. Rather, the tumor growth rate is significantly slowed, indicating that the T-antigen C-terminal region contributes to tumor progression in this cell type. In contrast, the pRB-binding region appears essential for tumorigenesis as mutation of residue 107, known to disrupt pRB and p107 binding to wild-type T antigen, abolishes the ability of the dl1137 protein to induce growth abnormalities in the brain

Vectors in p53 gene therapy



Non-viral vectors

- liposome
- Dendrimer
- Nano carrier

http://clincancerres.aacrjournals.org

investigations on P53 gene therapy

Type of vectors	year	Related articles
liposome	2014	A cationic cholesterol based nanocarrier for the delivery of p53-EGFP-C3 plasmid to cancer cells
	2006	p53 gene therapy of human osteosarcoma using a transferrin-modified cationic liposome
	2004	p53 and PTEN/MMAC1/TEP1 Gene Therapy of Human Prostate PC-3 Carcinoma Xenograft, Using Transferrin-Facilitated Lipofection Gene Delivery Strategy
	2002	Gene Therapy with P53 and a Fragment of Thrombospondin I Inhibits Human Breast Cancer in Vivo

Non-viral vectors(liposome)

Mol Cancer Ther 2005;4(4). April 2005

p53 gene therapy of human osteosarcoma using a transferrin-modified cationic liposome

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Non-viral vectors(liposome)

Abstract

Gene delivery via transferrin receptors, which are highly expressed by cancer cells, can be used to enhance the effectiveness of gene therapy for cancer. In this study, we examined the efficacy of *p53* gene therapy in human osteosarcoma (HOSM-1) cells derived from the oral cavity using a cationic liposome supplemented with transferrin. HOSM-1 cells were exposed to transferrin-liposome-*p53* *in vitro*, and the growth inhibition rate, expression of p53 and bax, and induction of apoptosis were measured 48 hours later. Treatment of HOSM-1 cells with transferrin-liposome-*p53* resulted in 60.7% growth inhibition. Wild-type p53 expression and an increase in bax expression were observed following transfection with transferrin-liposome-*p53*, and 20.5% of the treated HOSM-1 cells were apoptotic. *In vivo*, the HOSM-1 tumor transplanted into nude mice grew to 5 to 6 mm in diameter. Following growth of the tumor to this size, transferrin-liposome-*p53* was locally applied to the peripheral tumor (day 0) and then applied once every 5 days for a total of six times. During the administration period, tumor growth did not occur, and the mean tumor volume on the last day of administration (day 25) was 10.0% of that in the saline control group. These results suggest that *p53* gene therapy via cationic liposome modification with transferrin is an effective strategy for treatment of osteosarcoma.

[Mol Cancer Ther 2005;4(4):625–31]

Non-viral vectors(liposome)

Hum Gene Ther. 2002 Apr 10;13(6):761-73.

p53 and *PTEN/MMAC1/TEP1* Gene Therapy of Human Prostate PC-3 Carcinoma Xenograft, Using Transferrin-Facilitated Lipofection Gene Delivery Strategy

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ABSTRACT

We previously reported that supplementation of a cationic liposome with transferrin (Tf) greatly enhanced lipofection efficiency (P.-W. Cheng, Hum. Gene Ther. 1996;7:275-282). In this study, we examined the efficacy of *p53* and *PTEN* tumor suppressor gene therapy in a mouse xenograft model of human prostate PC-3 carcinoma cells, using a vector consisting of dimyristoyloxypropyl-3-dimethylhydroxyethyl ammonium bromide (DMRIE)-cholesterol (DC) and Tf. When the volume of the tumors grown subcutaneously in athymic nude mice reached 50-60 mm^3, three intratumoral injections of the following four formulations were performed during week 1 and then during week 3: (1) saline, (2) DC + Tf + pCMVlacZ, (3) DC + Tf + pCMVPTEN, and (4) DC + Tf + pCMV*p53* (standard formulation). There was no significant difference in tumor volume and survival between group 1 and group 2 animals. As compared with group 1 controls, group 3 animals had slower tumor growth during the first 3 weeks but thereafter their tumor growth rate was similar to that of the controls. By day 2 posttreatment, group 4 animals had significantly lower tumor volume relative to initial tumor volume as well as controls at the comparable time point. Also, animals treated with *p53* survived longer. Treatment with DC, Tf, pCMV*p53*, DC + pCMV*p53*, or Tf + pCMV*p53* had no effect on tumor volume or survival. Expression of *p53* protein and apoptosis were detected in tumors treated with the standard formulation, thus associating *p53* protein expression and apoptosis with efficacy.
However, *p53* protein was expressed in only a fraction of the tumor cells, suggesting a role for bystander effects in the efficacy of *p53* gene therapy. We conclude that intratumoral gene delivery by a nonviral vector consisting of a cationic liposome and Tf can achieve efficacious *p53* gene therapy of prostate cancer

investigations on P53 gene therapy

Type of vector	year	Related articles
Dendrimers	2013	Poly (amido amine) dendrimer silences the expression of epidermal growth factor receptor and p53 gene in vitro
	2013	Efficient Delivery of p53 and Cytochrome C by Supramolecular Assembly of a Dendritic Multi-Domain Delivery System

Non viral vectors(Dendrimer)

Efficient Delivery of p53 and Cytochrome C by Supramolecular Assembly of a Dendritic Multi-Domain Delivery System

- David Yuen Wah Ng,
- Jörg Fahrer,
- Yuzhou Wu,
- Klaus Ei

Abstract

Versatile nanocarrier systems facilitating uptake of exogenous proteins are highly alluring in evaluating these proteins for therapeutic applications. The self-assembly of an efficient nano-sized protein transporter consisting of three different entities is presented: A streptavidin protein core functioning as an adapter, second generation polyamidoamine dendrons for facilitating cell uptake as well as two different therapeutic proteins (tumor suppressor p53 or pro-apoptotic cytochrome c as cargo). Well-defined dendrons containing a biotin core are prepared and display no cytotoxic behavior upon conjugation to streptavidin. The integration of biotinylated human recombinant p53 (B-p53) into the three component system allows excellent internalization into HeLa, A549 and SaOS osteosarcoma cells monitored via confocal microscopy, immunoblot analysis and co-localization studies. In addition, the conjugation of B-p53 to dendronized streptavidin preserves its specific DNA-binding in vitro, and its delivery into SaOS cells impairs cell viability with concomitant activation of caspases 3 and 7. The versatility of this system is further exhibited by the significant enhancement of the pro-apoptotic effects of internalized cytochrome c which is analyzed by flow cytometry and cell viability assays. These results demonstrate that the "bio-click" self-assembly of biotinylated dendrons and proteins on a streptavidin adapter yields a stable supramolecular complex. This efficient bionanotransporter provides an attractive platform for mediating the delivery of functional proteins of interest into living mammalian cells in a facile and rapid way

Non viral vectors(Dendrimer)

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Poly (amido amine) dendrimer silences the expression of epidermal growth factor receptor and p53 gene *in vitro*

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Accepted 13 February, 2012

To understand more about the lower generations of poly(amido amine) dendrimer (PAMAM) as a non-viral vector for antisense (ANS) therapy, a 21-mer epidermal growth factor receptor (EGFR) ANS was delivered by generation five of PAMAM in T47D breast carcinoma cells in culture. The semi-quantitative polymerase chain reaction (PCR) and western blot analysis were used to quantify the expression of EGFR mRNA and protein, respectively. The results showed that PAMAM G5/ANS nanoparticles were able to decrease the level of EGFR mRNA more than 40% even at the lower dendrimer primary amine to the antisense phosphate groups (N/P) ratio of 0.5. But, only the data of western blot analysis at the higher N/P ratios of 10 and 20 showed a decrease of the protein expression level similar to the mRNA expression level. Moreover, PAMAM dendrimer had a positive effect on the EGFR ANS action to inhibit the EGFR mRNA and protein expression. Further studies revealed that PAMAM G5 dendrimer as such inhibits the expression of EGFR in a concentration-dependent manner. Since PAMAM as such was able to inhibit the mRNA expression of p53 gene, we speculated that the effect of PAMAM G5 on the EGFR is a kind of its non-selective effect on the transcription and/or translation machinery of the cell.

Key words: Poly(amido amine) dendrimer (PAMAM) dendrimer, epidermal growth factor receptor (EGFR) antisense, epidermal growth factor, RNAi, polyamidoamine dendrimer, toxicogenomics, gene delivery.

investigations on P53 gene therapy

Type of vectors	year	Related articles
Nano carrier	2016	p53 gene therapy of human breast carcinoma: using a transferrin-modified silica nanoparticles
	2015	Oral nano-delivery of anticancer ginsenoside 25-OCH3-PPD, a natural inhibitor of the MDM2 oncogene: Nanoparticle preparation characterization, in vitro and in vivo anti-prostate cancer activity, and mechanisms of action
	2015	combination wt-p53 and microRNA 125b transfection in a genetically engineered lung cancer model using dual cd44/EFGR-targeting nanoparticles
	2008	Novel cationic solid lipid nanoparticles enhanced p53 gene transfer to lung cancer cells

Non viral vectors(Nano carrier)

January 2016, Volume 23, Issue 1, pp 101–110

p53 gene therapy of human breast carcinoma: using a transferrin-modified silica nanoparticles

Background

Nanoparticles have an enormous potential for development in biomedical applications, such as gene or drug delivery. In our study, we examined the efficacy of p53 gene therapy in human breast carcinoma (MCF-7) cells using silica nanoparticles (SiNPs) supplemented with transferrin.

Methods

MCF-7 cells were exposed to transferrin–SiNPs–p53 in vitro, and the growth inhibition rate, expression of p53 and bax, and induction of apoptosis were measured 48 h later.

Results

Treatment of MCF-7 cells with transferrin–SiNPs–p53 resulted in 60.7 % growth inhibition. Wild-type p53 expression and an increase in bax expression were observed following transfection with transferrin–SiNPs–p53, and 20.5 % of the treated MCF-7 cells were apoptotic. In vivo, the MCF-7 tumor transplanted into nude mice grew to 5–6 mm in diameter. Following growth of the tumor to this size, transferrin–SiNPs–p53 was locally applied to the peripheral tumor (day 0) and then applied once every 5 days for a total of six times. During the administration period, tumor growth did not occur, and the mean tumor volume on the last day of administration (day 25) was 10.0 % of that in the saline control group.

Conclusion

These results suggest that p53 gene therapy via transferrin-modified silica nanoparticles is an effective strategy for treatment of breast carcinoma.

Non viral vectors(Nano carrier)

www.impactjournals.com/oncotarget/ Oncotarget, Vol. 6, No. 25

Oral nano-delivery of anticancer ginsenoside 25-OCH_3-PPD, a natural inhibitor of the MDM2 oncogene: Nanoparticle preparation, characterization, *in vitro* and *in vivo* anti-prostate cancer activity, and mechanisms of action

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Keywords: *molecular targeting efficiency, MDM2, ginsenoside, PEG-PLGA nanoparticles, oral delivery*

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Non viral vectors(Nano carrier)

ABSTRACT

The Mouse Double Minute 2 (*MDM2*) oncogene plays a critical role in cancer development and progression through p53-dependent and p53-independent mechanisms. Both natural and synthetic MDM2 inhibitors have been shown anticancer activity against several human cancers. We have recently identified a novel ginsenoside, 25-OCH_3-PPD (GS25), one of the most active anticancer ginsenosides discovered thus far, and have demonstrated its MDM2 inhibition and anticancer activity in various human cancer models, including prostate cancer. However, the oral bioavailability of GS25 is limited, which hampers its further development as an oral anticancer agent. The present study was designed to develop a novel nanoparticle formulation for oral delivery of GS25. After GS25 was successfully encapsulated into PEG-PLGA nanoparticles (GS25NP) and its physicochemical properties were characterized, the efficiency of MDM2 targeting, anticancer efficacy, pharmacokinetics, and safety were evaluated in *in vitro* and *in vivo* models of human prostate cancer. Our results indicated that, compared with the unencapsulated GS25, GS25NP demonstrated better MDM2 inhibition, improved oral bioavailability and enhanced *in vitro* and *in vivo* activities. In conclusion, the validated nano-formulation for GS25 oral delivery improves its molecular targeting, oral bioavailability and anticancer efficacy, providing a basis for further development of GS25 as a novel agent for cancer therapy and prevention.

Non viral vectors(Nano carrier)

Novel cationic solid lipid nanoparticles enhanced p53 gene transfer to lung cancer cells

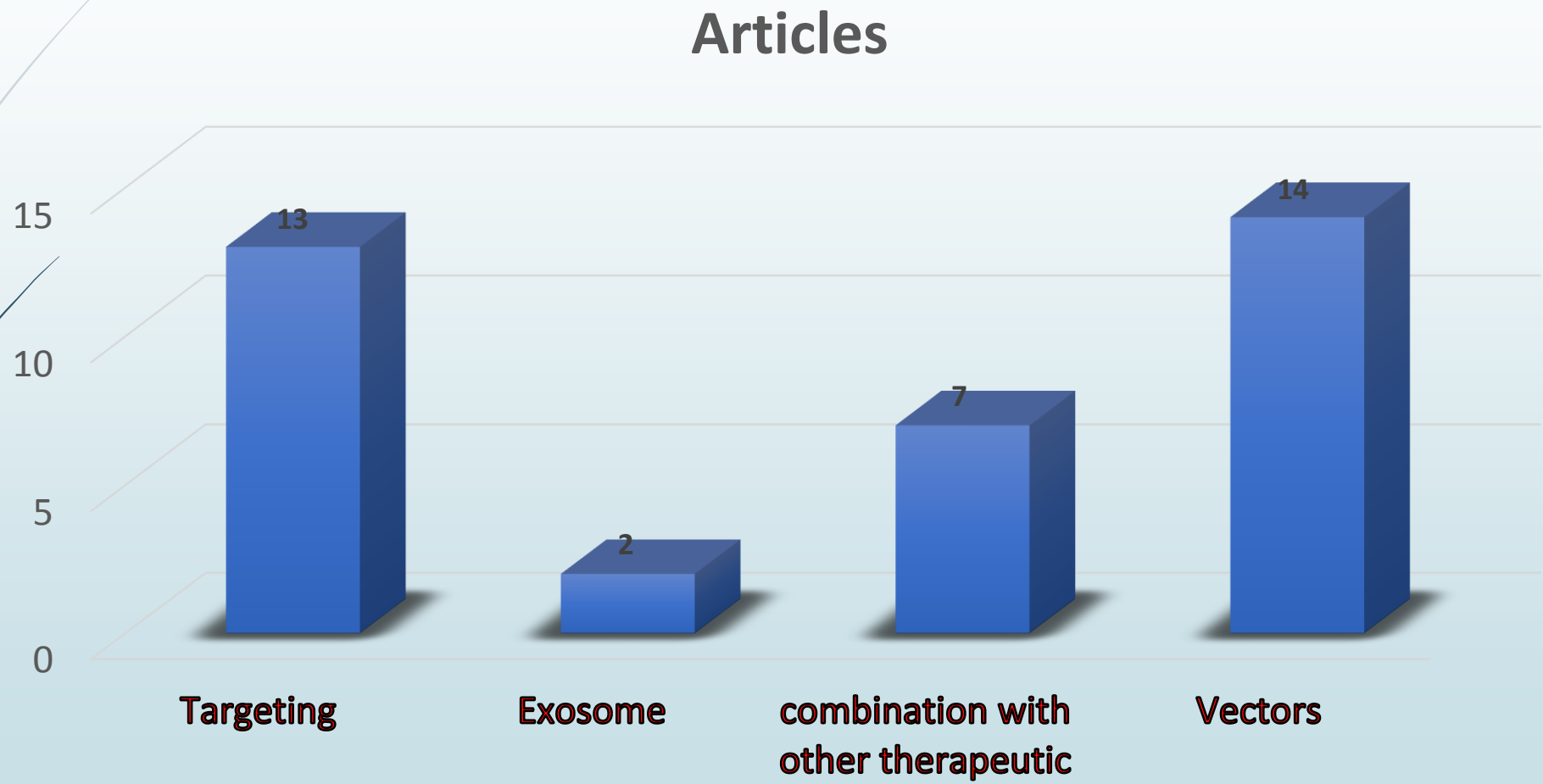
- Sung Hee Choi[a], Su-Eon Jin[a], Mi-Kyung Lee[b], Soo-Jeong Lim[c], Jeong-Sook Park[a],
- Byung-Gyu Kim[c], Woong Shick Ahn[c], Chong-Kook Kim[a]

Abstract

Mutations in the p53 tumor suppressor gene are the most common molecular genetic abnormalities to be described in lung cancer. However, there have been few reports of nonviral vector-mediated p53 gene delivery in lung cancer. A new formulation of cationic solid lipid nanoparticles (SLNs) for gene delivery was produced by the melt homogenization method with slight modification, and the SLNs were formulated by mixing tricaprin (TC) as a core, 3β[*N*-(*N'*, *N'*-dimethylaminoethane) carbamoyl] cholesterol (DC-Chol), dioleoylphosphatidylethanolamine (DOPE) and Tween 80 in various ratios. Plasmid DNA (pp53-EGFP)/SLNs complexes were transfected into human non-small cell lung cancer cells (H1299 cells) and transfection efficiency was determined by FACS analysis. The gene expression was determined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis. The cellular growth inhibition and apoptosis of treated cells with pp53-EGFP/SLNs complexes were assessed by trypan blue exclusion assay and annexin V staining, respectively. *In vivo* biodistribution of plasmid DNA was investigated by PCR and RT-PCR. The transfection efficiency of SLN1 (TC:DC-Chol:DOPE:Tween 80 = 0.3:0.3:0.3:1), which showed the highest transfection efficiency among the SLN formulations, was higher than that of commercially available Lipofectin®. The SLNs-mediated transfection of the p53 gene resulted in efficient high levels of wild-type p53 mRNA and protein expression levels in H1299 cells. The efficient reestablishment of wild-type p53 function in lung cancer cells restored the apoptotic pathway. Taken together, our results reveal that cationic SLN-mediated p53 gene delivery may have potential for clinical application as a nonviral vector-mediated lung cancer therapy due to its effective induction of apoptosis and tumor growth inhibition


When?
Where?
Conclusion
Who?
Who?

p53 gene therapy/different types



Google scholar & www.ncbi.com /
last update:10 April 2017

Conclusion points

1) in the near future, gene therapy will take its place alongside chemotherapy, radiotherapy, and surgery as one of the tools routinely available to help treat patients with cancer.

2) TP53 gene therapy has been tested in clinical trials in patients with lung cancer, head and neck cancer, prostate, cervical &breast cancer and other tumors and showed hopeful results.

3) studies have demonstrated a significant clinical effect with stabilized tumor growth or even tumor regression in at least a fraction of the treated patients, and no major toxicity

4) additional clinical data is necessary to verify if these investigations can effectively activate p53 and improve the clinical efficacy in patients carrying wild-type p53

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The best way
to predict the future
is to create it.
Peter Drucker